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Repeatability of 2 Methods for Assessment of Insulin Sensitivity and Glucose Dynamics in Horses

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Both the euglycemic-hyperinsulinemic clamp (EHC) and minimal model analysis of the frequently sampled intravenous glucose tolerance test (FSIGT) have been applied for measurement of insulin sensitivity in horses. However, no published data are available on the reproducibility of these methods. Therefore, the objective of this study was to evaluate the variation and repeatability of measures of glucose dynamics and insulin sensitivity in horses derived from minimal model analysis of the FSIGT and from the EHC method. Six healthy horses underwent both the FSIGT and EHC on 2 occasions over a 4-week period, with a minimum of 5 days between tests. Coefficient of variation (CV) and intraclass correlation coefficient (ICC) were calculated for measures of glucose metabolism and insulin sensitivity derived from each test. In the EHC, insulin sensitivity, expressed as the amount of metabolized glucose (M) per unit of serum insulin (I) (M/I ratio), averaged $0.19 \pm 0.06 \times 10^{-4}$ mmol/kg/min·(pmol/L)$^{-1}$ with an average interday CV of 14.1 ± 5.7% (range, 7–20%) and ICC of 0.74. Minimal model analysis of the FSIGT demonstrated mean insulin sensitivity (Si) of $0.49 \pm 0.17 \times 10^{-4}$/min × (pmol/L)$^{-1}$ with an average interday CV of 23.7 ± 11.2% (range, 9–35%) and ICC of 0.33. Mean CV and ICC for minimal model glucose effectiveness (Sg) and acute insulin response (AIRg) were, respectively, 26.4 ± 11.2% (range 13–40%) and 0.10 and 11.7 ± 6.5% (range 7–21%) and 0.98. Insulin sensitivity measured by the EHC has lower interday variation when compared with the minimal model estimate derived from the FSIGT.

Key words: Euglycemic-hyperinsulinemic clamp; Glucose tolerance test; Minimal model analysis.

Insulin resistance (IR), a pathologic condition in which the magnitude of the biological response to insulin is decreased,[1] is of interest because it has been associated with several disease states of horses, including obesity, laminitis, osteochondritis dissecans, hyperlipemia, and hyperadrenocorticism.[2–5] However, the role of IR in the pathogenesis of these conditions is uncertain, in part because most studies have used indirect measures of insulin resistance, such as oral or intravenous glucose-tolerance tests. In human medicine, several methods have been used for determination of insulin sensitivity, including the euglycemic-hyperinsulinemic clamp (EHC), insulin tolerance test, insulin suppression test, minimal model analysis of a frequently sampled intravenous glucose tolerance test (FSIGT), and homeostasis model assessment (HOMA).[6,7] Of these, the EHC and minimal model analysis of the FSIGT are the most well-established methods for accurate quantitative assessment of insulin action.[6,7] The euglycemic clamp method involves induction and maintenance of hyperinsulinemia by a continuous infusion of insulin, while the blood glucose concentration is held constant at basal concentration by a variable glucose infusion using a negative-feedback principle. Under steady-state conditions of euglycemia and hyperinsulinemia, endogenous glucose production is suppressed and the glucose infusion rate equals glucose uptake by all tissues in the body and is a measure of tissue sensitivity to exogenous insulin.[8]

Mathematical modeling of plasma glucose and insulin concentrations during a FSIGT offers an alternative to the glucose clamp for quantifying insulin sensitivity. In humans, dogs, and cats, measures of insulin sensitivity obtained from the minimal model are closely correlated with measures obtained with the glucose clamp technique.[9,10] The minimal model method has several advantages when compared with the glucose clamp method, including ability to obtain estimates of both insulin-dependent and insulin-independent glucose utilization as well as a measure of the sensitivity of pancreatic β cells to glucose. However, the mathematical models are based on many assumptions about insulin and glucose kinetics, which may result in systematic errors in the estimates of glucose- and insulin-dependent glucose utilization. In addition, this test requires a large insulin response to obtain a precise estimate of insulin sensitivity, limiting its use in patients with pancreatic β-cell insufficiency.[11]

Both the glucose clamp and minimal model techniques have been applied in horses.[3,12] However, with the exception of 1 report in which a single horse underwent the glucose clamp on 3 occasions,[13] no published data exist on the reproducibility of these measures of insulin sensitivity. A priori knowledge of the test variability will assist researchers in the interpretation of test results for the assessment of glucose dynamics and insulin sensitivity and is needed for calculation of sample sizes for clinical or experimental studies. Therefore, the primary objective of our study was to determine the variability and repeatability of measures of insulin sensitivity and glucose metabolism derived from the euglycemic-hyperinsulinemic clamp and from minimal model analysis of the FSIGT.

Materials and Methods

Horses

Six clinically healthy Standardbred horses (1 gelding, 5 mares; 5–14 years of age, mean ± SD body weight 481 ± 50 kg) were used. None of the mares demonstrated clinical signs of estrus on test days. All horses were judged to be in moderate body condition; the range in body condition score was 4–7.[14] Horses were fed long-stem grass

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hay from a single batch (dry matter, 87.1%; crude protein, 10.4% [dry matter basis]; nonstructural carbohydrate, <3%) at approximately 2% body weight/d. The ration was divided into 2 equal portions, fed at 0700 and 1400 hours. Horses were housed in box stalls with free-choice access to water and trace-mineral salt and provided access to a small pasture 2 hours daily between 0900 and 1500 hours. Each horse underwent both the EHC and FSIGT on 2 occasions over a 4-week period, with a minimum of 5 days between tests. The ordering of tests was randomized such that the 1st procedure was either a EHC or a FSIGT. Before the study, horses were acclimated to prolonged (3-hour) restraint in stocks on 3 occasions. The study was conducted in August and September 2002. All procedures were approved by the University of Guelph's Animal Care Committee and performed in compliance with their recommendations.

Experimental Procedures

For all testing procedures, feed was withheld beginning at 1800 hours the night before the test, and tests commenced at 0700 hours. For the glucose clamp, horses stood in stocks for the entire procedure, whereas, for the FSIGT, horses stood in stocks for the 1st 40 minutes of the test, after which the horses were moved to 3.5 × 3.5 m box stalls for the remainder of the protocol. The horses were loosely restrained to prevent access to water and bedding. Catheters (14 gauge, 5½ in) were inserted into the left and right jugular veins for, respectively, blood sampling and infusion (dextrose and insulin). The catheters were inserted after aseptic preparation and analgesia of the overlying skin and were connected to extension lines and fastened to the skin by use of sutures and adhesive. Blood samples were collected at various times during each test (see below). Aliquots of samples were placed into tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant or no additive. The EDTA samples were kept on ice for approximately 20 minutes and centrifuged for 10 minutes at 1,500 × *g* to separate plasma. The tubes without additive were allowed to clot at room temperature, after which the samples were centrifuged for separation of the serum. Plasma and serum samples were stored at −20°C until analysis for glucose and insulin concentrations, respectively.

Euglycemic-Hyperinsulinemic Clamp

On the morning of the clamp procedure, the insulin infusate was prepared by mixing 2 mL of each horse's serum (to prevent adsorption of insulin to plastic surfaces) with 5 mL of human recombinant DNA insulin (100 U/mL)[a] and 493 mL of 0.9% NaCl. After collection of baseline blood samples, infusion of insulin via a peristaltic pump[b] at a rate of 21.3 pmol/min/kg (3 mU/min/kg) body weight (bwt) was started and maintained for the 180-minute procedure. Blood samples (~2 mL) were drawn at 5-minute intervals throughout the clamp for determination of glucose concentration by a precision glucometer.[c] The glucometer measures whole blood glucose concentration by the glucose dehydrogenase method and has an intraassay coefficient of variation of <2% for equine blood. However, blood glucose concentrations measured by use of the precision glucometer consistently are 0.6–0.7 mmol/L lower when compared with plasma glucose concentrations determined by a spectrophotometric method (unpublished data). A calibrated syringe pump was used to deliver a variable rate infusion[d] of glucose (50% w/v dextrose[e]) for maintenance of euglycemia, defined as a blood glucose concentration of 5 mmol/L. When blood glucose deviated by more than 0.2 mmol/L from euglycemia, the glucose infusion rate was adjusted empirically based on the method of DeFronzo.[8] Additional blood samples (~10 mL) for determination of serum immunoreactive insulin were obtained every 15 minutes.

The 1st 90 minutes of the clamp was considered an equilibration period, with data from the final 60 minutes used for calculation of the rate of whole-body glucose uptake (*M*) and an index of insulin sensitivity (glucose uptake per unit of insulin [*I*], *M/I*). For these calculations, it is assumed that endogenous glucose production is completely suppressed by hyperinsulinemia such that *M* is equal to the amount of glucose infused.[8] The validity of this assumption in horses has not been determined. Because glucose is not maintained perfectly constant during the clamp, a correction (the space correction [*SC*]) is required to account for glucose that has been added or removed from the glucose space other than by metabolism. The *SC* (mmol/kg/min) was calculated from the equation

$$SC = (G_2 - G_1)(0.19 \text{ bwt})/(T \times \text{bwt}),$$

where G_2 and G_1 are glucose concentrations at the beginning and end of the time interval, *T* is the time interval (5 minutes), bwt is body weight in kg, and the term (0.19 × bwt) is the glucose space in liters. The *M* (mmol/kg/min) was calculated by use of the following equation:

$$M = GIR - SC,$$

where *GIR* is the glucose infusion rate (mmol/kg/min) and *SC* is the space correction.

The steady-state insulin concentration (*I*), taken as the average of serum insulin concentrations measured during the final 60 minutes of the clamp, was used to calculate the mean *M/I* ratio. This ratio, which reflects the rate of glucose disposal per unit of insulin, is an index of tissue sensitivity to exogenous insulin.[8,13]

Frequently Sampled Intravenous Glucose Tolerance Test

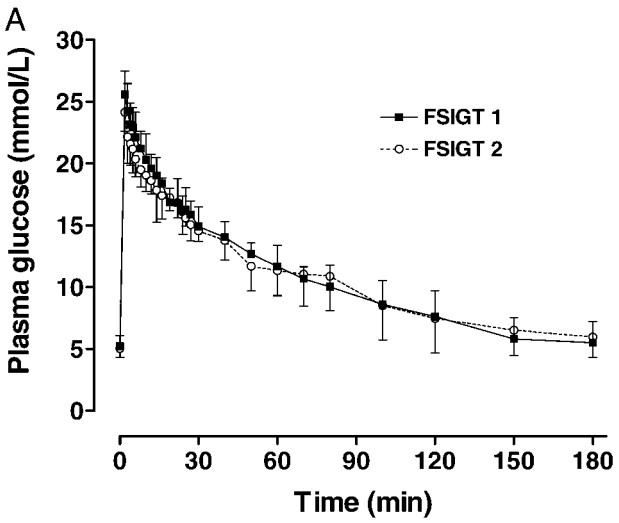
Five minutes after collection of a blood sample for determination of basal insulin and glucose concentrations, dextrose[e] (50% w/v; 0.5 g/kg) was administered intravenously. Subsequent blood samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 minutes after dextrose administration. Glucose concentration was measured in all samples, whereas serum insulin concentration was measured at 0, 2, 4, 6, 8, 10, 14, 18, 20, 30, 60, 90, 120, 150, and 180 minutes.

Two main approaches were used to mathematically describe responses in plasma glucose and serum insulin during the FSIGT. First, the glucose and insulin concentration data were analyzed by the minimal model method, which provides an estimation of the dynamic relation between the time course of the changes in glucose and insulin after an intravenous glucose load. This analysis, performed using a computerized algorithm,[f] provides 3 primary variables: (1) the insulin sensitivity index, S_I, which reflects the change in net fractional glucose clearance rate per unit change in insulin after intravenous glucose administration; (2) glucose effectiveness, S_G, which represents the net fractional glucose clearance rate independent of insulin; and (3) the acute (1st 20 minutes after glucose administration) insulin response to glucose (AIRg), calculated by computation of the incremental area under the insulin curve. Second, the incremental areas under the concentration versus time curves (AUC) for both glucose (AUCg) and insulin (AUCi) were calculated by the trapezoidal method,[g] and the plasma half-life for glucose clearance (T½g) was estimated by use of nonlinear regression analysis of glucose concentration versus time between 1 and 180 minutes after glucose administration.[g]

Blood analyses—Plasma glucose concentrations were measured in duplicate by use of an enzymatic spectrophotometric method.[h] The mean intraassay and interassay coefficient of variation (CV) were both <3%. Serum immunoreactive insulin concentrations were measured in duplicate by use of a commercial solid-phase radioimmunoassay[i] that has been validated for equine serum.[15] The mean intraassay and interassay CV were 5.5% and 9.6%, respectively.

Statistical Analysis

Normality of distribution of the data were tested using the Shapiro-Wilk statistic. Non-Gaussian distributed data were log 10 transformed



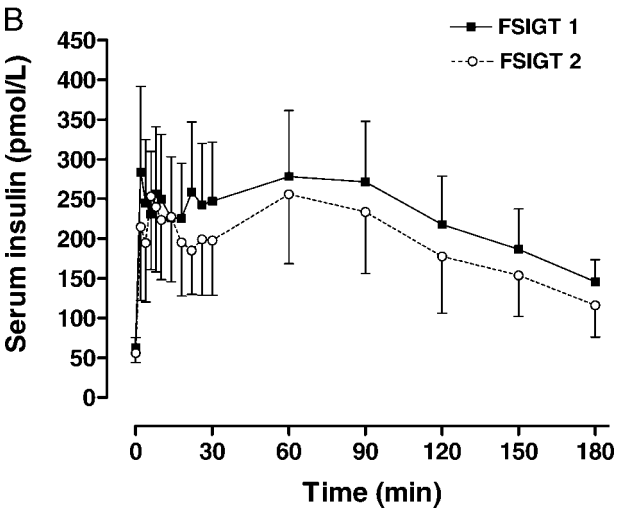


Fig 1. Time course of plasma glucose (**A**) and serum insulin (**B**) concentrations for the 2 frequently sampled glucose tolerance tests (FSIGT). Data are means ± SD for 6 horses.

to achieve normality. Measurement error, defined as the variation between measurements of the same quantity on the same animal, was quantified in 2 ways. First, the intraclass correlation coefficient (ICC), which represents an estimate of the average correlation between all possible ordering of pairs of measures, was calculated.[16] Second, the within-subject standard deviation was calculated from the formula $s_w = \frac{1}{2}n\Sigma d^2i$, where n is the number of horses, d is the difference between the 2 observations, and d^2i is the variance term for the 2 observations on horse i. The repeatability coefficient of the measurements then was calculated as $2.77s_w$, wherein the difference between 2 measurements for the same horse is expected to be less than $2.77s_w$ for 95% of pairs of observations.[17] Intertest variation in dependent variables also was expressed as the CV, calculated as the standard deviation (SD) divided by the mean of each set of 2 tests. The resultant value was multiplied by 100. The results of repeated tests were compared by use of a paired t-test or 2-way (time and test as independent variables) repeated measures analysis of variance (ANOVA).[J] Significance was accepted at $P < .05$. Values are reported as means and SDs unless stated otherwise.

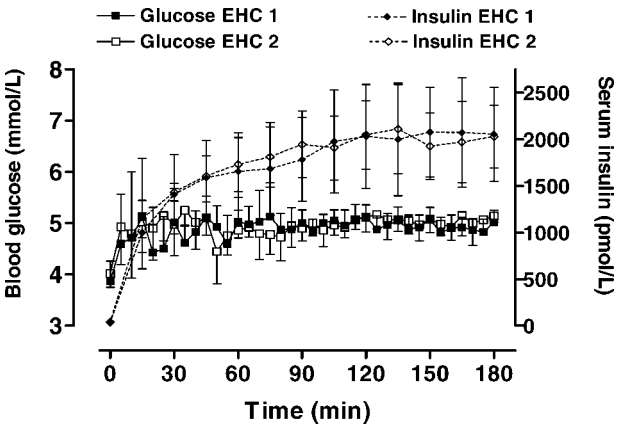


Fig 2. Blood glucose and serum insulin concentrations during the two 180-minute euglycemic-hyperinsulinemic clamp (EHC) procedures. Data are means ± SD for 6 horses.

Results

Mean values for plasma glucose and serum insulin concentrations during the 2 FSIGT tests are depicted in Figure 1A,B. Plasma glucose concentrations at all time points did not differ between the 2 tests (Fig. 1A). Peak glucose concentration (1 minute postinjection) was similar in test 1 (25.3 ± 1.78 mmol/L) and 2 (24.9 ± 1.53 mmol/L) and, for both procedures, plasma glucose concentration at 180 minutes was not different when compared with baseline. Serum insulin concentrations also followed a similar pattern in the 2 FSIGT procedures, and no difference was observed between tests at any time point (Fig. 1B).

In both EHC procedures, a progressive increase in serum insulin concentration occurred during the 1st 90 minutes, with a plateau evident between 105 and 180 minutes (Fig. 2). Mean serum insulin concentrations during the final 60 minutes (ie, 5 measurements) of EHC 1 and EHC 2 were 2067 ± 379 and 2031 ± 425 pmol/L, respectively, with an associated mean CV of 3.9 ± 1.3% and 4.2 ± 1.9%. Similarly, whole-blood glucose concentration was stable throughout the final 90 minutes of the clamp. The overall mean blood glucose concentrations in EHC 1 and EHC 2 during this period were 4.9 ± 0.23 and 5.0 ± 0.19 mmol/L, respectively. The corresponding mean CVs were 2.3 ± 0.5% and 2.1 ± 0.4%. The glucose space corrections for EHC 1 and EHC 2 were 0.11 ± 0.20 and 0.06 ± 0.10 × 10^{-2} liters, respectively.

Calculated variables from the FSIGT and EHC procedures are shown in Table 1. Mean glucose half-life (T½g), AUCg, S_I, S_G, AIRg, M, and M/I did not differ between tests 1 and 2. However, mean AUCi was significantly greater in test 1 than in test 2. Minimal model analysis of the FSIGT indicated an overall mean S_I of 0.49 ± 0.17 × 10^{-4}/min·(pmol/L)$^{-1}$, whereas the overall mean M/I for the EHC was 0.19 ± 0.06 × 10^{-4} mmol/kg/min·(pmol/L)$^{-1}$. Test-retest variability in calculated variables was assessed using CV, whereas repeatability was evaluated using the ICC and the repeatability coefficient ($2.77s_w$). The minimal model estimates of insulin sensitivity (S_I) and glucose-mediated glucose disposal (S_G) demonstrated moderately high variability (mean of individual CVs of 23% and 26%, respec-

Table 1. Mean (± SD) values for calculated variables and measures of variability and repeatability for frequently sampled glucose tolerance test and euglycemic-hyperinsulinemic clamp procedures in 6 horses.

Variable	Test 1	Test 2	Mean CV % (range)	ICC	Repeatability ($2.77s_w$)
AUCg (mmol/L/180 min)	1,965 ± 340	2,061 ± 457	19.2 ± 6.0 (11.6–29.7)	0.63	397.6
AUCi (pmol/L/180 min)	27,344 ± 8,256	23,059 ± 8,172*	23.7 ± 12.7 (7.6–51.4)	0.72	843.8
T½g (minute)	30.4 ± 9.0	31.1 ± 10.6	11.3 ± 9.4 (3.5–27.7)	0.81	7.7
S_I ($\times 10^{-4}$/min · [pmol/L]$^{-1}$	0.471 ± 0.165	0.508 ± 0.195	23.7 ± 11.2 (8.9–34.5)	0.33	0.376
S_G (min^{-1})	1.77 ± 0.90	1.41 ± 0.47	26.4 ± 10.1 (13.0–39.6)	0.10	1.80
AIRg (pmol × min/L)	1,355 ± 1,155	1,216 ± 1,150	11.7 ± 6.5 (6.7–21.3)	0.98	124.9
M (mmol/kg/min)	0.0349 ± 0.010	0.0388 ± 0.012	12.2 ± 10.5 (1.2–30.4)	0.73	0.0149
M/I ($\times 10^{-4}$ mmol/kg/min · [pmol/L]$^{-1)}$	0.180 ± 0.005	0.197 ± 0.007	14.1 ± 5.7 (7.0–19.8)	0.74	0.0831

AUCg, area under the glucose versus time curve; AUCi, area under the insulin versus time curve; T½g, glucose half-time; S_I, insulin sensitivity index; S_G, glucose effectiveness; AIRg, acute insulin response to glucose; *M*, rate of glucose metabolism; *M/I*, ratio of metabolized glucose to steady-state insulin concentration; CV, coefficient of variation; ICC, intraclass correlation coefficient; s_w, measurement error.

* $P < .05$ versus Test 1.

tively) and poor repeatability, as demonstrated by an ICC of 0.33 and 0.10 for S_I and S_G, respectively. Furthermore, the differences between the 2 measurements were $>2.77s_W$ for all horses. On the other hand, AIRg had lower variability (CV ~ 12%) and an ICC of 0.98, but the difference between the 2 measurements was $<2.77s_W$ in only 3 of 6 horses. The ICC for glucose half-time (T½g), AUCg, and AUCi all were >0.60, but the difference between 2 measurements was $<2.77s_W$ in only 1 of 6 horses for AUCi and 2 of 6 horses for T½g.

Measures of glucose disposal (*M*) and insulin sensitivity (*M/I* ratio) during the EHC generally showed lower variability and higher repeatability in comparison with variables derived from the FSIGT. The mean CVs for *M* and *M/I* ratio were, respectively, 12.2 ± 10.5% and 14.1 ± 5.7%, with corresponding ICC of 0.73 and 0.74. The mean CVs were biased by the results for horse 2, in which *M* and *M/I* were markedly lower in EHC 1 when compared with EHC 2. This horse was restless and excitable during the 1st clamp but demonstrated calm demeanor during the 2nd procedure, and it therefore is possible that this difference in demeanor contributed to the wide variation between EHC 1 and EHC 2. When the data from horse 2 were excluded from the analysis, the mean CV for *M* and *M/I* ratio decreased to 5.7 ± 4.0% and 12.1 ± 5.0%, respectively. The difference between the 2 measurements of *M/I* ratio was $<2.77s_W$ for all horses. For *M*, this criterion was achieved for 5 of 6 horses. Overall, therefore, the best combination of test characteristics (low mean of individual CVs, small repeatability coefficient, high ICC) was observed for *M* and *M/I* derived from the EHC.

Discussion

The primary objective of this study was to determine the test-retest variability and repeatability in insulin sensitivity and glucose dynamics measured by the EHC and by minimal model analysis of the FSIGT. To our knowledge, the present study is the 1st to examine the variability and repeatability of these procedures in horses. We observed that insulin sensitivity measured by the EHC (the *M/I* ratio) had a mean CV of approximately 14%, whereas S_I measured by the FSIGT had a mean CV of approximately 24%. Therefore, under the conditions of the present study, the EHC method appeared to be more repeatable than minimal model analysis of the FSIGT for determination of insulin sensitivity in horses.

Several statistical methods have been used to assess the repeatability or reproducibility of diagnostic tests applied to the same individual on more than 1 occasion. These include correlation coefficients, paired *t*-test, limits of agreement, ICC, and the repeatability coefficient.[18,19] Although correlation coefficients frequently are used to describe the repeatability of tests, difficulties exist in interpretation in part because the strength of the relationship depends on the order in which the data are entered.[17] The ICC avoids this problem because it computes the average correlation among all possible orderings of pairs and, for this reason, we chose to use the ICC for assessment of repeatability. However, both the Pearson's *r* and ICC are highly sensitive to sample heterogeneity (ie, with a varied sample, the likelihood of obtaining a high value of *r* is high). Therefore, to broaden the assessment of repeatability, we also calculated the repeatability coefficient, which is derived from the within-subject standard deviation (s_W) of repeated measurements. Using this assessment, a repeatable test is one in which the difference between 2 measurements for the same subject is expected to be $<2.77s_W$ for 95% of pairs of observations.

Variation in glucose dynamics and insulin sensitivity can be attributed to 2 components. First, measurement errors arise from errors in sample timing and the measurement of plasma glucose and insulin concentrations. Second, true fluctuations in glucose- and insulin-mediated glucose metabolism may occur. The influence of these 2 components on the dependent variables is difficult to establish. However, with regard to estimation of S_I, statistical modeling experiments (Monte Carlo simulation) demonstrated that a 1.5% glucose assay error and an 8% insulin assay error (similar to the assay variance observed in our laboratory) can account for approximately 12% of the variance, with the remaining variability attributed to true interday variation in insulin-mediated glucose disposal.[20] In this context, the present study was designed to minimize such interday var-

iation by control of environmental influences on insulin sensitivity. First, the study was performed under conditions of stable diet and controlled physical activity. Second, before the study, horses were acclimated to prolonged restraint in stocks in an attempt to minimize day-to-day variation in excitement. Stress and excitement, with attendant increases in the circulating concentrations of the counterregulatory hormones (eg, epinephrine, cortisol), can markedly decrease the precision of measures of glucose dynamics and insulin sensitivity.[21] As mentioned, excitement is 1 possible explanation for the higher variability of *M* and *M/I* in horse 2 in comparison with the other horses.

The EHC has been used in several studies of horses,[12,13,22] but only 1 of these reports provided data on the reproducibility of results. Rijnen and coworkers[13] administered the EHC in 1 horse on 3 occasions over a 3-week period, although the 1st test used a lower priming insulin dose when compared with later procedures. In this horse, close agreement was observed among the 3 tests for *M* values (0.013, 0.016, and 0.016 mmol/kg/min) but wider variation occurred in the *M/I* ratio with mean values of 0.00037, 0.00057, and 0.00067 $\times$ 100 mmol/kg/min·(pmol/L)$^{-1}$. In studies of healthy human subjects, the mean CV for average glucose disposal rate (*M*) during an EHC of similar duration (3 hours) to the present investigation was approximately 15% in 1 study[23] and 10% in another.[24] These values are similar to those observed in the present study of 12.2 and 14.1% for glucose disposal rate (*M*) and the ratio of glucose disposal rate to steady-state insulin concentration ratio (*M/I*), respectively. The moderately high ICC ($>$0.70) and small repeatability coefficients for these measures also indicated acceptable repeatability.

The intravenous glucose tolerance test (IVGTT) traditionally has been used for assessment of glucose tolerance in horses. However, to date, few studies in horses have provided quantitative data describing glucose and insulin responses during the IVGTT. In the present study, we administered the standard glucose dose of 0.5 g/kg bwt but, unlike the standard IVGTT, used a frequent sampling protocol during the 1st hour that permitted minimal model analysis and quantitative description of glucose dynamics and insulin sensitivity. Hoffman and colleagues[3] recently have reported on the use of minimal model analysis of the FSIGT in horses. However, the protocol employed by Hoffman et al.[3] differed from that of the present study in that a lower dose of glucose (0.3 g/kg bwt) was administered and exogenous insulin (30 mU/kg) was given 20 minutes after the glucose. These differences in methodology preclude direct comparison of the data.

The variability in minimal model insulin sensitivity (S_I) and glucose effectiveness (S_G) observed in the present study is comparable with that reported in studies of human subjects[20] and cats.[21] For example, in healthy men subjected to 3 FSIGTs over 12 days, the average interday CVs for S_I and S_G were 20.2% (range 6–44%) and 25.1% (range 6.2–105%), respectively.[20] In another study, the mean interday CVs for S_I and S_G were 16.9% and 16.6%.[25] In cats, the mean CVs for S_I and S_G were 35.4% and 24.7%, respectively.[21] Importantly, however, the aforementioned studies did not report repeatability coefficients or similar valid statistics describing repeatability of the minimal model estimates. In the current study, the low ICC and high value for the repeatability coefficient for S_I suggest inferior repeatability of the minimal model method when compared with the EHC for assessment of insulin sensitivity in horses.

Variability in the insulin response after glucose injection is one possible explanation for the higher variability of the minimal model insulin sensitivity measure when compared with estimates derived from the EHC. Although the insulin response during the 10-minute period after glucose injection (AIRg) was highly repeatable with a mean CV of 12% and an ICC of 0.98, the overall insulin response (AUCi) during the 180-minute FSIGT demonstrated higher test-retest variation (CV $\sim$ 24%) and lower repeatability, with statistically higher AUCi in FSIGT 1 than in FSIGT 2. The variation in insulin response in the horses of the present study is similar to that reported in humans[26] and cats.[26] Importantly, studies of the minimal model in humans have demonstrated that the dynamics of the insulin pattern affects the precision of estimates of S_I. In particular, S_I is more precisely estimated when there is a sharp, well-defined 2nd-phase insulin response.[27,28] For this reason, a modified FSIGT protocol was developed in which insulin or tolbutamide (an insulin secretatogue) is administered 20 minutes after glucose injection. This approach has resulted in improved test performance, including decreased within-subject variation and enhanced correlation between minimal model S_I and the euglycemic clamp.[11] As mentioned, the insulin-modified FSIGT recently has been applied to the horse,[3] but no published data are available on the repeatability of this method. Future studies should address this issue.

Mean CV for glucose half-time (T½g) in our study ($\sim$11%) was lower than that reported for cats ($\sim$20%)[26] and humans ($\sim$21%).[29] However, the low repeatability of the T½g, as indicated by the large value of the repeatability coefficient, lessens confidence in the utility of this measure for assessment of glucose tolerance.

In humans, the precision of the minimal model estimate of insulin sensitivity is lower in patients with marked insulin resistance (eg, non–insulin-dependent diabetes mellitus [NIDDM]). In 1 study of NIDDM patients,[30] the mean CV of S_I was 105%, considerably higher than the range of CVs (14–30%) reported for healthy humans.[11,30] Furthermore, the correlation between minimal model estimates of insulin sensitivity and those from the euglycemic clamp is much weaker in insulin-resistant when compared with healthy human subjects.[9,31] Clinically healthy horses were used in the present study and, therefore, our data concerning the repeatability of the EHC and minimal model may not be applicable to horses with insulin resistance.

In summary, the results of the present study demonstrate that the EHC method is more repeatable than minimal model analysis of the FSIGT for determination of insulin sensitivity in horses. As such, the EHC may be better suited for longitudinal studies that examine the effects of disease state or environmental modification (eg, diet, physical conditioning) on insulin sensitivity in horses. Future studies should examine the repeatability of the insulin-modified FSIGT for estimation of insulin sensitivity in horses and determine the accuracy of estimates of insulin sensitivity derived from single measurements of plasma glucose and

insulin concentrations (eg, HOMA and the Quantitative Insulin Sensitivity Check Index).

Footnotes

[a] Humulin R, Ely Lily, Indianapolis IN
[b] Vet IV 2.2, Heska Corporation, Denver, CO
[c] Accusoft, Roche Diagnostics, Ontario, Canada
[d] Precision Syringe Pump, KD Scientific, Kansas City, MO
[e] 50% Dextrose, Vedco Inc, Ontario, Canada
[f] MinMod Millenium, v. 5.15 (courtesy of Dr Ray Boston, University of Pennsylvania)
[g] GraphPad 3.0, Prizm, San Diego, CA
[h] Thermo Trace Ltd, Melbourne, Australia
[i] Coat-a-Count, DPC, Los Angeles, CA
[j] Sigmastat 3.0, SPSS Inc, Chicago, IL

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