Use of proxies and reference quintiles obtained from minimal model analysis for determination of insulin sensitivity and pancreatic beta-cell responsiveness in horses

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Objective—To develop proxies calculated from basal plasma glucose and insulin concentrations that predict insulin sensitivity (SI; L•min^{-1}•mU^{-1}) and beta-cell responsiveness (ie, acute insulin response to glucose [AIRg]; mU/L•min^{-1}) and to determine reference quintiles for these and minimal model variables.

Animals—1 laminitic pony and 46 healthy horses.

Procedure—Basal plasma glucose (mg/dL) and insulin (mU/L) concentrations were determined from blood samples obtained between 8:00 AM and 9:00 AM. Minimal model results for 46 horses were compared by equivalence testing with proxies for screening SI and pancreatic beta-cell responsiveness in humans and with 2 new proxies for screening in horses (ie, reciprocal of the square root of insulin [RISQI] and modified insulin-to-glucose ratio [MIRG]).

Results—Best predictors of SI and AIRg were RISQI (r = 0.77) and MIRG (r = 0.75) as follows: SI = 7.93(RISQI) – 1.03 and AIRg = 70.1(MIRG) – 13.8, where RISQI equals plasma insulin concentration$^{-0.5}$ and MIRG equals [800 – 0.30(plasma insulin concentration – 50)^2]/(plasma glucose concentration – 30). Total predictive powers were 78% and 80% for RISQI and MIRG, respectively. Reference ranges and quintiles for a population of healthy horses were calculated nonparametrically.

Conclusions and Clinical Relevance—Proxies for screening SI and pancreatic beta-cell responsiveness in horses from this study compared favorably with proxies used effectively for humans. Combined use of RISQI and MIRG will enable differentiation between compensated and uncompensated insulin resistance. The sample size of our study allowed for determination of sound reference range values and quintiles for healthy horses. (*Am J Vet Res* 2005;66:2114–2121)

Changes in insulin sensitivity are associated with certain diseases, including some forms of exertional rhabdomyolysis, osteochondrosis, hyperadrenocorticism, and related syndromes, such as hyperlipidemia and laminitis.[1-6] Insulin sensitivity is also an important component of energy regulation during exercise, pregnancy and lactation, aging, and obesity.[7-9,10] Specific quantitative methods for determining insulin resistance are limited by their technical complexity and expense[11,12] and have been applied only to studies[5,8,9,10,13] of obesity, reproduction, exercise, and polysaccharide storage myopathy in the horse. Other common, nonspecific indications of insulin resistance, such as fasting hyperinsulinemia and glucose intolerance, are ambiguous and unstandardized.[13]

Simple, single sample predictors of insulin sensitivity based on basal plasma concentrations of insulin and glucose have been used as surrogates and proxies in human studies.[14-21] Plasma samples for determination of basal glucose and insulin concentrations may provide good representations of the glucose-insulin system and predictors of disease because they describe the chronic unperturbed state of the subject. Reference quintiles for parameters of insulin resistance further allow for comparisons of individual data collected from a large population. The combined use of proxies and reference quintiles facilitates the diagnosis and characterization of clinical cases.

The purpose of the study reported here was to apply the minimal model of glucose and insulin dynamics to 4 groups of healthy horses. Proxies calculated from plasma samples for determination of basal plasma concentrations of glucose and insulin were compared with parameters of the minimal model in terms of concordance and predictive power, and reference quintiles were defined for both types of indices. Data from 1 hyperlipidemic laminitic pony, the first such clinical case in which insulin sensitivity has been quantified by the minimal model, and from 1 horse for which the minimal model was unable to estimate SI have been used to illustrate the value of simple tests and reference ranges. Some of the data have been published.[10]

Materials and Methods

Animals—The 46 healthy horses used in our study included 10 mature Thoroughbred geldings (tested in August 2002), 12 pregnant Thoroughbred mares (tested in April 2003), 12 Arabian geldings (tested in October and November 2003), and 12 Thoroughbred weanlings (tested in November 2002). Geldings were a mean ± SD of 12 ± 3 years old (range, 9 to 18 years old) with body condition scores of 5 to 8 (on a scale of 1 to 9).[9] Pregnant mares were at 299 ± 3 days of gestation and were 11 ± 4 years old (range, 6 to 17 years old) with body condition scores of 4.5 to 5. Arabians were 11 ± 1

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years old (range, 5 to 16 years old) with body condition scores of 4 to 7. Weanlings were 199 ± 5 days old with body condition scores of 5 or 6.[10] All horses were maintained on mixed grass–legume pasture and supplemented twice daily with feed high in sugar and starch, resembling a typical commercial sweet feed, or feed high in fat and fiber.[9] Supplements have been described previously[9,10] and provided approximately a third or half of daily energy requirements.[22] Data were also obtained from 1 hyperlipidemic laminitic pony[a] and 1 obese insulin-resistant Thoroughbred gelding.

Procedures—Basal plasma concentrations of glucose (mg/dL) and insulin (mU/L) were determined from the mean value of 2 or 3 baseline samples taken from each horse prior to conducting a **frequent sampling IV glucose tolerance test (FSIGT)** for the minimal model. Horses were kept in stalls overnight and had free access to grass hay and water but no concentrate. Blood samples for determination of basal plasma concentrations of glucose and insulin were taken between 8:00 AM and 9:00 AM for all groups. Plasma glucose concentrations were determined by use of an enzymatic assay.[b] Plasma insulin concentrations were determined by use of a radioimmunoassay[c] previously validated for equine insulin.[23] The intra-assay **coefficients of variation (CVs)** of duplicate samples were < 1% for glucose and 5% for insulin.

Results of the FSIGT for each horse were analyzed according to the minimal model of glucose and insulin dynamics.[24] The model is used to calculate values for the **insulin sensitvity index (SI;** units) **SI** ($L \cdot min^{-1} \cdot mU^{-1}$), **glucose effectiveness** (**Sg**; min^{-1}), **acute insulin response to glucose** (**AIRg**; $mU/L \cdot min^{-1}$), and **disposition index** (**DI**; dimensionless). The model effectively characterized the 45 horses and the hyperlipidemic laminitic pony but found no solution for 1 obese Thoroughbred gelding. Acronyms for proxies and equations used for their calculation are provided (**Appendix**).

Statistical analysis—Correlation coefficients and linear regressions were used to compare proxies and determine the best-fitting equations to predict SI, Sg, AIRg, and DI from proxies. Concordance with the appropriate minimal model parameter (SI or AIRg) was calculated for each proxy according to Lin.[25] Equivalence was tested by use also of lines of identity and Bland-Altman plots.[26,27]

Data sets for healthy horses were tested for normalcy by use of the Shapiro-Wilk statistic, then used to calculate reference ranges,[28] and divided into quintiles.[29] Data in the lowest quintile of SI and AIRg were compared with corresponding values predicted by the **reciprocal inverse square of basal insulin (RISQI)** and the **modified insulin-to-glucose ratio (MIRG)**, respectively. Results were categorized as **true positive (TP)**, **false positive (FP)**, **true negative (TN)**, and **false negative (FN)**. Sensitivity was calculated as TP/(TP + FN), specificity was calculated as TN/(FP + TN), and total predictive value was calculated as (TP + TN)/(TP + FP + TN + FN).[30]

Results

The new proxies for screening SI and pancreatic beta-cell responsiveness in horses were compared with standard proxies for humans by use of correlation coefficients, Bland-Altman limits of agreement, and concordance coefficients with their respective minimal model parameters (**Table 1**). Regression of SI on RISQI (insulin concentration$^{-0.5}$) gave the best-fitting predictive equation for SI as follows (r = 0.774; n = 46; P < 0.001):

$$SI = (7.93 \pm 0.99)(RISQI) + (-1.03 \pm 0.41)$$

Similarly, the regression of AIRg on MIRG ([800 – 0.30{insulin concentration – 50)^2}/[glucose concentration – 30]) gave the best-fitting predictive equation for insulin secretory response as follows (r = 0.754; n = 46; P < 0.001):

$$AIRg = (70.1 \pm 9.2)(MIRG) + (-13.8 \pm 41.1)$$

No satisfactory regression was found for DI or Sg.

Reference ranges and reference quintiles for parameters of the minimal model and their proxies were calculated nonparametrically (**Table 2**). An example of graphic analysis for equivalence by use of RISQI is shown (**Figure 1**). The slope and intercept of the overall regression of SI for each proxy of insulin sensitivity were conserved in the 4 groups. No significant (P = 0.28) difference in regression slopes was found between groups adapted to diets rich in sugar and starch or fat and fiber.

The predictive power of proxies calculated from basal plasma concentrations of glucose and insulin was also analyzed in terms of assessing the lowest quintile of SI from the population (**Figure 2**). All proxies of SI had similar specificity (approx 85%), sensitivity

Table 1—Analyses of proxies compared with minimal model parameters of insulin resistance and beta-cell function.

Comparisons*	Correlation coefficient	Bland-Altman 95% limits of agreement†	Concordance coefficient‡
Compared with SI			
Basal insulin concentration	–0.521	± 4.71	0.427
HOMA	–0.502	± 4.96	0.402
QUICKI	0.738	± 2.63	0.705
Basal glucose-to-insulin ratio	0.758	± 2.47	0.730
RISQI	0.774	± 2.36	0.749
Compared with AIRg			
Insulin-to-glucose ratio	0.725	± 323	0.689
HOMA-BC%	0.736	± 334	0.703
MIRG	0.754	± 306	0.725

*All relationships were significant (P < 0.001). †Calculated as $2 \times SD_{difference\ in\ observed}$.[26] ‡Evaluation of the degree to which coordinate pairs (observed, predicted) fall on the line of identity.[25]

SI = Insulin sensitivity index. HOMA = Homeostasis model assessment. QUICKI = Quantitative insulin sensitivity check index. RISQI = Reciprocal of the square root of insulin. AIRg = Acute insulin response to glucose. HOMA-BC% = Percent HOMA–beta-cell function. MIRG = Modified insulin-to-glucose ratio.

Table 2—Summary statistics and reference ranges for minimal model parameters and basal properties.

Variable	Mean	Median	95% reference interval*	Quintiles 1	2	3	4	5
SI × 10^4 ($L•min^{-1}•mU^{-1}$)	2.09	1.82	0.16–5.88	0.14–0.78	0.79–1.50	1.51–2.27	2.28–3.04	3.05–5.94
Sg × 10^2 (min^{-1})	0.01	0.0095	0.12–2.95	0.09–0.72	0.73–0.88	0.89–1.28	1.29–1.92	1.92– 2.96
AIRg ($mU/L•min^{-1}$)	270	218	67–805	60–148	149–190	191–273	274–337	338–808
DI × 10^4	479	381	39.3–1,675	30–207	208–316	317–427	428–817	818–1,752
Basal insulin (mU/L)	9	7.9	1.22–40.40	1.10–4.53	4.54–6.48	6.49–8.93	8.94–11.46	11.47–43.29
Basal glucose (mg/dL)	101.7	97.8	73.9–124.7	73.2–90.4	90.5–94.8	94.9–101.2	101.3–119.5	119.6–124.9
RISQI $(mU/L)^{-0.5}$	0.392	0.356	0.159–0.917	0.152–0.295	0.296–0.335	0.336–0.393	0.394–0.470	0.471–0.953
MIRG ($mU_{insulin}^2/[10•L•mg_{glucose}]$)	4.05	4.18	1.24–10.26	1.20–2.12	2.13–3.48	3.49–4.54	4.55–5.27	5.27–10.67

*Confidence intervals determined nonparametrically as the 2.5th to 97.5th percentile.[44]
DI = Disposition index. Sg = Glucose effectiveness.
See Table 1 for remainder of key.

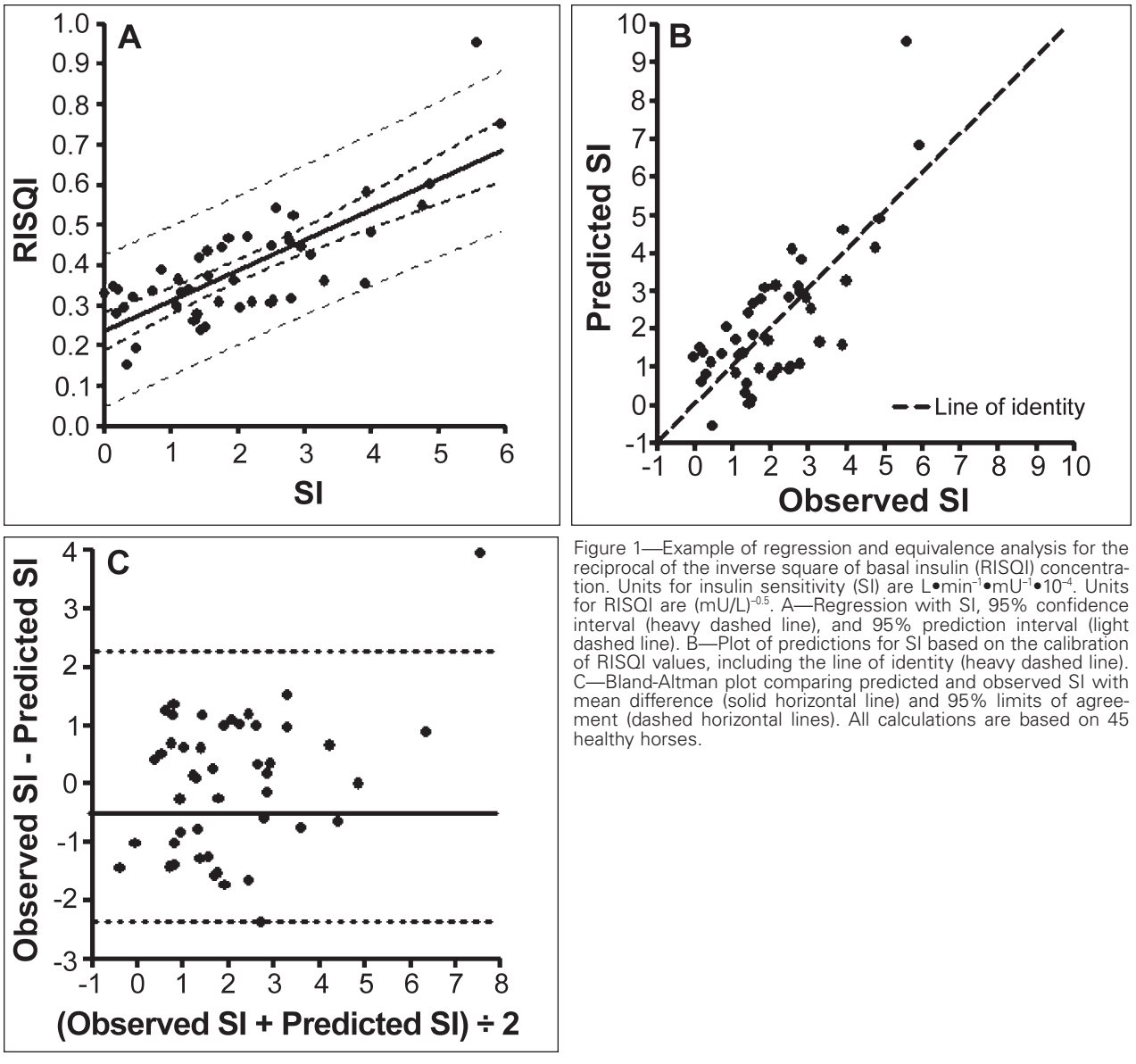

Figure 1—Example of regression and equivalence analysis for the reciprocal of the inverse square of basal insulin (RISQI) concentration. Units for insulin sensitivity (SI) are $L•min^{-1}•mU^{-1}•10^{-4}$. Units for RISQI are $(mU/L)^{-0.5}$. A—Regression with SI, 95% confidence interval (heavy dashed line), and 95% prediction interval (light dashed line). B—Plot of predictions for SI based on the calibration of RISQI values, including the line of identity (heavy dashed line). C—Bland-Altman plot comparing predicted and observed SI with mean difference (solid horizontal line) and 95% limits of agreement (dashed horizontal lines). All calculations are based on 45 healthy horses.

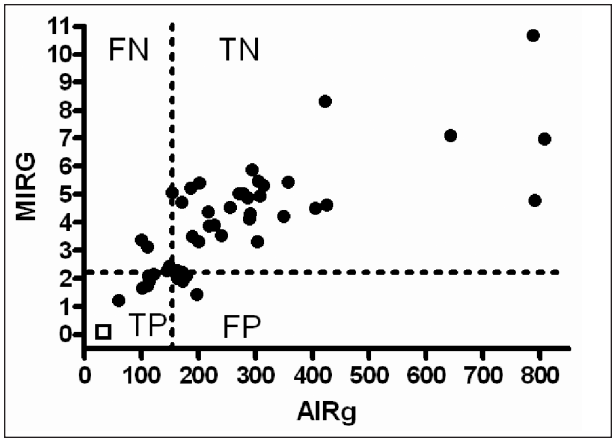

Figure 2—Plot of modified insulin-to-glucose ratio (MIRG) versus beta-cell response (ie, acute insulin response to glucose [AIRg]) for 46 healthy horses (closed circles) and 1 hyperlipidemic laminitic pony (open square). Cutoff value for poor insulin responders is determined by the lowest quintile of AIRg (vertical dashed line). Cutoff value for poor insulin responders is determined by the lowest quintile of MIRG (horizontal dashed line). Points in each quadrant represent horses with results in the following respective categories: true positive (TP; n = 5), false positive (FP; 4), false negative (FN; 4), or true negative (TN; 33). These numbers of horses are used to calculate sensitivity, specificity, and total predictive value of MIRG.

(approx 45%), and total predictive power (approx 78%). The same was true for all proxies of AIRg, which had specificities of approximately 88%, sensitivities of approximately 50%, and total predictive power of approximately 80%.

Data for the laminitic pony were as follows: SI $\times 10^4 = 0.089$ L•min^{-1}•mU^{-1}, Sg $\times 10^2 = 1.37$ min^{-1}, AIRg = 35 mU/L•min^{-1}, DI $\times 10^4 = 3$, basal insulin concentration = 101.3 mU/L, basal glucose concentration = 170.7 mg/dL, RISQI = 0.099 (mU/L)$^{-0.5}$, and MIRG = 0.075 $mU_{insulin}^2/(10 \bullet L \bullet mg_{glucose})$. Reference quintiles were expressed graphically and used to characterize deviations of the laminitic pony (**Figure 3**). The insulin sensitivity of the laminitic pony was in the lowest quintile of SI and RISQI, and despite high basal insulin secretion, this pony had an MIRG and AIRg in the lowest quintile. A basal plasma insulin concentration of 48.38 mU/L, a basal plasma glucose concentration of 112.34 mg/dL, an RISQI of 0.144 (mU/L)$^{-0.5}$, and an MIRG of 9.71 $mU_{insulin}^2/(10 \bullet L \bullet mg_{glucose})$ were found for the obese gelding that had no estimate of minimal model parameters.

Discussion

Results of our study indicate that reference quintiles and proxies for screening insulin sensitivity and pancreatic beta-cell responsiveness in healthy horses can be obtained from calculated parameters of the minimal model. Correlations of the new proxies for screening insulin sensitivity and pancreatic beta-cell responsiveness in horses with minimal model parameters of insulin sensitivity and AIRg compare favorably with the best proxies for humans that have been used successfully in public health.[14-21] Combined use of RISQI and MIRG, which represent insulin sensitvity and insulin response, will enable assessment of compensatory insulin secretion in apparently healthy horses and insulin signaling failure in hyperglycemic horses.

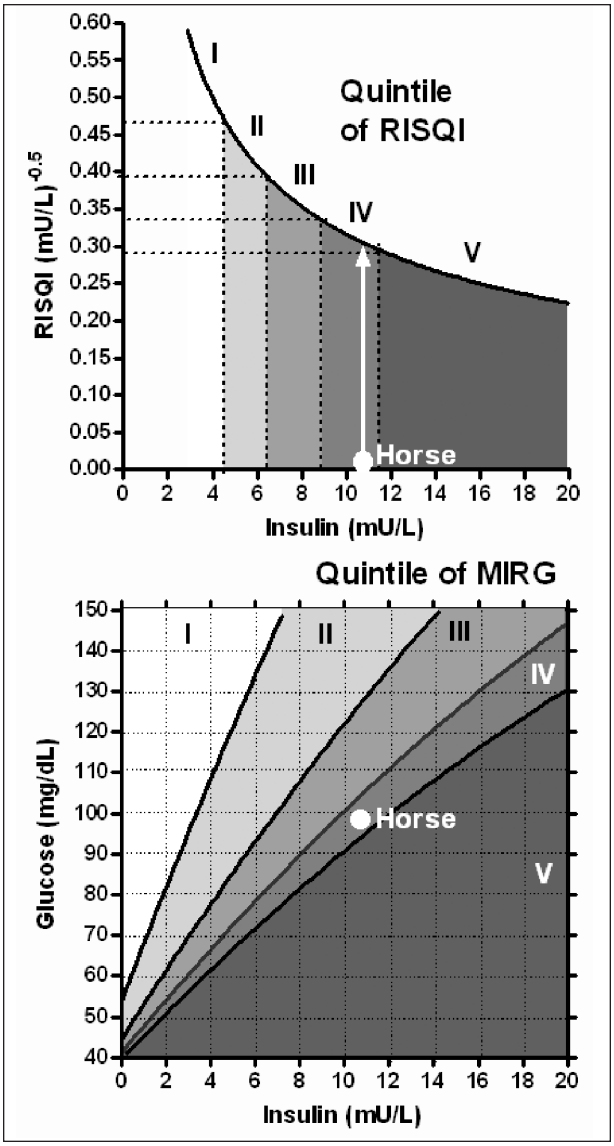

Figure 3—Plots of basal plasma concentrations of glucose and insulin to determine reference quintiles for a healthy horse. Top—Quintiles of SI are determined by plotting basal plasma insulin concentration versus RISQI. Basal plasma insulin concentration of the horse was 10.7 mU/L. Notice that the horse has an SI within the fourth quintile (60th to 80th percentile). Bottom—Quintiles of MIRG are determined by plotting basal plasma insulin concentration versus basal plasma glucose concentrations. Basal plasma glucose concentration of the horse was 98.8 mg/dL. Notice that the horse has an MIRG within the fourth quintile.

Proxies are less accurate than the specific quantitative parameters they predict. However, proxies identify well-studied properties of insulin resistance and regulation with statistically determined power and are therefore superior to nonspecific indications, such as basal hyperinsulinemia, glucose intolerance, or analogies to diseases in other species.[12] In comparison to specific quantitative tests, proxies calculated from basal plasma concentrations of glucose and insulin greatly increase the efficiency of resource use for a more extensive generation of information. Proxies calculated in this manner have been useful in human research on large populations and in clinical situations requiring convenient

and cost-effective evaluations of insulin resistance[14-21] and should be equally useful in equine studies.

Insulin sensitivity in the minimal model (SI, $L \bullet min^{-1} \bullet mU^{-1}$) represents insulin-mediated glucose disposal or the rate of glucose clearance from plasma ($L \bullet min^{-1}$) per unit of plasma insulin (mU).[31] Proxies for SI are based on plasma insulin and plasma glucose concentrations. The correlation coefficient (r = 0.774) of RISQI and SI may be compared with corresponding proxies used in studies of public health (r = 0.60, **homeostasis model assessment [HOMA]**[14]; r = 0.73, glucose-to-insulin ratio[20]; r = 0.50, fasting insulin[20]; r = 0.89, HOMA and the **quantitative insulin sensitivity check index [QUICKI]**[32]; r = 0.91, glucose-to-insulin ratio[32]; r = 0.53, HOMA[33]; and r = 0.52, QUICKI[15]).

Fasting hyperinsulinemia has been used previously as an indicator of insulin resistance in horses and humans.[1,7,34] This association is attributed to increased secretion of insulin by pancreatic beta cells to compensate for decreased insulin sensitivity. In humans, fasting plasma insulin concentrations may decline as the beta cells fail. This decompensation defines the advent of type 2 diabetes mellitus. Decompensation may also occur in the diabetic-like condition of late pregnancy, which is associated with pregnancy loss in women and perhaps mares.[7,34,35]

Fasting hyperglycemia is an unreliable index for insulin resistance because plasma glucose concentration is maintained in a limited range by strong glucose homeostasis involving several regulators, including insulin. Our results indicate that a high basal plasma glucose concentration is not a common risk factor in healthy horses. Elevated basal plasma glucose concentration is more likely to be a sign of current disease, such as hyperlipidemia, which is common in ponies,[1] or possibly type 2 diabetes mellitus, which is infrequently observed in horses.[36-38]

From similar results in the equivalence tests, the proxies for SI can be categorized into 2 groups: those with basal insulin concentrations in the numerator (HOMA and basal insulin concentration) and those with insulin concentrations in the denominator (QUICKI, the basal glucose-to-insulin ratio, and RISQI). Proxies obtained by calculations with insulin concentration in the numerator had lower correlation and concordance and higher Bland-Altman limits of agreement than proxies obtained by calculations with insulin concentration in the denominator. This is largely the result of several horses with low SI and high basal insulin concentrations. In humans, high insulin secretion is generally preempted by beta-cell failure, where insulin secretion reaches a limit; at this point, hyperglycemia and type 2 diabetes mellitus ensue.[26] The premise behind HOMA was to take advantage of this reciprocal relationship between basal insulin and glucose concentrations to avoid the ambiguity of low insulin concentrations that could indicate high insulin sensitivity or failed insulin secretion.[39] In humans, the HOMA index has shown good correlation to the euglycemic-hyperinsulinemic clamp technique and, to a lesser extent, the hyperglycemic clamp technique, although its precision was poor.[31] The HOMA index has also been shown to correlate with SI determined from the minimal model.[14] In the horse, however, beta cells appear particularly resistant, and in insulin-resistant horses, high basal insulin concentrations are commonly observed, as was the case for several horses in our study, whereas type 2 diabetes mellitus rarely occurs.[36-38] As a result, proxies obtained from calculations with insulin concentration in the denominator (QUICKI, basal glucose-to-insulin ratio, and RISQI) would be expected to more accurately estimate insulin sensitvity with a positive correlation and high insulin values normalized to the remaining data.

The QUICKI was developed in humans as a simple method to assess insulin sensitivity as determined by the hyperinsulinemic-euglycemic clamp technique and is based on the same reciprocal relationship between insulin and glucose as the HOMA.[15] The QUICKI has been shown to improve precision in insulin sensitivity estimation, compared with the HOMA index in adult human subjects,[15,16] but this improvement is inconsistent in children and adolescents.[17,18] In humans (including nonobese, obese, and diabetic subjects), QUICKI was shown to correlate better with insulin sensitivity, compared with HOMA, as determined by the minimal model and the glucose clamp technique.[15] Results from the present study also show improved precision with the use of QUICKI, compared with HOMA.

The basal glucose-to-insulin ratio is derived from a differing concept on the relationship between glucose and insulin, where the insulin-secreting response of the beta cells is exaggerated despite normal glucose concentrations. Therefore, a low glucose-to-insulin ratio indicates low insulin sensitivity. The glucose-to-insulin ratio was originally used as an indicator of polycystic ovary syndrome in women.[19,34] The ratio has since been shown to correlate with insulin sensitivity in women with polycystic ovary syndrome[20] and girls with premature adrenarche.[21] It has also been shown to increase precision in estimates of insulin sensitivity, compared with HOMA or QUICKI,[18,34] which was also the case in our study.

The QUICKI and basal glucose-to-insulin ratio include basal glucose in their assessment. In our study, basal glucose concentration had no relationship to insulin sensitivity and was not shown to substantially improve estimations of SI, compared with the reciprocal of basal insulin concentration alone. This finding reflects the large variation in basal insulin concentration (CV = 88%) relative to basal glucose concentration (CV = 15%). Results of human studies[39,40] have similar variation in basal glucose concentrations for healthy subjects but increased variability in diabetic subjects. As in humans, larger variation in basal glucose concentration may be expected in sick horses, in which case the glucose-to-insulin ratio and QUICKI may provide additional information, compared with RISQI. However, we recommend comparing 2 proxies, RISQI and MIRG, that separate insulin sensitivity and insulin secretion and avoid ambiguous determinations of the dynamic glucose and insulin system. Also, the simpler test with use of only basal insulin concentration is preferable, as glucose sample collection and analysis add an extra source of error.

The insulin response to a glucose challenge is measured for the first 10 minutes of the minimal model and defines the acute (phase 1) response of insulin to glucose (AIRg).[31] This measurement of insulin secre-

tion has been considered an estimate of beta-cell activity.[41,42] High values of AIRg have been associated with low SI and described as a compensation for insulin inefficiency. Low values of AIRg in conjunction with hyperglycemia indicate a state of decompensation in which beta cells fail to provide adequate insulin to maintain glucose homeostasis. All proxies for AIRg are based on the insulin-to-glucose ratio, which estimates the amount of insulin secretion stimulated per unit of circulating glucose.

The correlation coefficient (r = 0.754) of MIRG and AIRg may be compared with corresponding proxies used in a study[32] of public health (r = 0.60, **percent HOMA–beta-cell function** [**HOMA-BC%**]; r = –0.66, glucose-to-insulin ratio). The HOMA estimate of beta-cell function was first proposed by Matthews et al[39] and was correlated to beta-cell functions estimated by use of the hyperglycemic clamp technique, IV glucose tolerance test, and continuous glucose infusion model assessment. The HOMA-BC% was shown to correlate slightly (r = 0.58) to AIRg in human children and adolescents.[17] The HOMA-BC% was also shown to correlate well to first-phase insulin response in groups of people with varying glucose tolerance and fasting hyperglycemia, but no consistent relationship was observed across the groups.[33]

The insulin-to-glucose ratio closely resembles the HOMA-BC% and is an adaptation of the insulinogenic index, which is the ratio of insulin-to-glucose 30 minutes after an oral glucose tolerance test.[43,44] Single sample proxies derived from oral tolerance tests are highly speculative because ingestion, digestion, and absorption can vary dramatically between subjects. Nevertheless, the insulinogenic index has been correlated to the first-phase insulin response to IV administration of glucose.[17,45] A fasting version of the insulinogenic index has also been shown to correlate to the first- and second-phase insulin response during the hyperglycemic clamp technique but only to the second-phase insulin response in women with polycystic ovary syndrome.[18] Results of human studies indicate that surrogate estimates of beta-cell function are adequate for groups with similar health status but not across groups, so proxies for beta-cell function are evaluated carefully for characterizing disease states.[18,33] In our study, MIRG successfully predicted AIRg for the healthy population and the hyperlipidemic laminitic pony.

Together, RISQI and MIRG identify apparently healthy individuals that are compensating for low SI with increased beta-cell activity. The combination also allows for assessment of the ability of an individual to tolerate increases in plasma glucose that might be encountered following meals of concentrated feeds, when grazing rich pasture, or during veterinary treatment. In addition, these proxies provide a means to determine whether changes in glucose tolerance are occurring between plasma insulin and its target action at the level of the tissue or whether changes result from altered beta-cell responsiveness. This can then identify affected horses where insulin administration is indicated or when various management strategies will be effective.

Reference ranges are usually based on 95% confidence intervals of the sample distribution from approximately 50 healthy animals so that appropriate determinations of normalcy can be achieved.[45] Reference ranges for insulin resistance have been proposed on the basis of data from 4 ponies and 5 horses,[46] despite the inadequacy of these small sample sizes. The meager number of observations in studies of horses with the glucose clamp technique[46] vitiates the power of such experiments and reinforces the need for proxies.[12,47]

The comparison between insulin sensitivity and response in individuals to a larger population of horses is facilitated by use of reference quintiles, especially when expressed graphically. Reference quintiles can be used to characterize individual deviations and to monitor the progress of an affected horse. For the laminitic pony[b] of our study, insulin sensitivity was in the lowest quintile of SI and RISQI, agreeing with the suggestion that insulin resistance implicated in the pathogenesis of hyperlipidemia and laminitis.[1] Despite a high basal insulin secretion, this pony had an MIRG in the lowest quintile, indicating a reduced capacity for beta-cell secretion. This decompensation was made evident by hyperglycemia (basal glucose concentration in the highest quintile) and indicates that this pony could benefit from exogenous insulin administration.

The obese Thoroughbred gelding provides another example of the use of proxies and reference quintiles. When adapted to the sugar and starch diet, data from the FSIGT provided no solution to the minimal model for this horse.[9] However, the RISQI of the gelding was the lowest observed in healthy horses, qualifying it for the lowest quintile of insulin sensitivity. This result is consistent with the association between obesity and insulin resistance.[9] Despite a low insulin sensitivity, the high MIRG of the gelding demonstrates effective compensation and glucose homeostasis was maintained. Diet and exercise management might benefit this horse. Indeed, adaptation of the gelding to a diet with a low glycemic index increased the RISQI by 11% and MIRG by 10%, suggesting improved insulin sensitivity and beta-cell function.

Reference quintiles and proxies for SI should be especially valuable in research on physiologic conditions (such as obesity, pregnancy, and exercise) and diseases (such as exertional rhabdomyolysis, osteochondrosis, hyperlipidemia, laminitis, and pituitary adenoma) that may be characterized by alterations in glucose and insulin regulation.[1-9,10]

For groups of horses, and perhaps for serial samples obtained while monitoring an affected horse, proxies calculated from basal plasma concentrations of glucose and insulin appear to be reliable predictors of corresponding sets of SI and AIRg data. This assertion is supported by strong correlations, tight confidence intervals, and conservation of regression lines between groups in our study.

The observed correlation coefficient of 0.744 for the association between RISQI and SI corresponds to a coefficient of determination of 0.55, or 55% of the variation in SI being accounted for by a variation in RISQI. Similarly, 57% of the variation in AIRg can be accounted for by variation in MIRG. In contrast, < 10% of the variation in Sg and < 20% of the variation in DI could be accounted for by variation in proxies obtained from basal insulin and glucose concentrations.

We found no human study of proxies for insulin sensitivity that performed Bland-Altman analysis to test equivalence. Simple correlation can be misleading, as correlations do not account for measurement bias and are influenced by the range of the data.[26] The Bland-Altman plot provides a quantitative analysis of the agreement between predicted and observed means while taking into consideration the variability in each. Calibration ensured no bias in the mean prediction for SI or AIRg by its respective proxies, compared with observed SI or AIRg. The prediction interval of the regression lines and the 95% limits of agreement of the Bland-Altman plots, however, revealed a degree of approximation for all proxies. For RISQI, 95% of estimates from the proxy should fall within 2.5 SI units of the actual SI. This imprecision indicates that proxies calculated from basal plasma concentrations of glucose and insulin should be used with caution when estimating individual values of SI.

The concordance correlation coefficient is another means to assess equivalence between 2 measurement techniques.[25] Concordance describes the agreement between paired measurements and their relation to the line of identity. Concordance values of > 0.7 indicate that the difference between the proxy estimates and their quantitative measurement was $< 30\%$ of the deviation expected for unrelated pairs of measurements.

The predictive power of proxies calculated from basal plasma glucose and insulin concentration was also analyzed in terms of assessing the lowest quintile from the population. Predictive power analysis has been used for previous comparisons of indices of insulin resistance.[17,19,20] Use of these indices allowed appropriate selection of $> 85\%$ of horses within the top 4 quintiles of SI but was only sensitive enough to identify approximately 45% of individuals within the lowest quintile of SI. Sensitivity should improve with a larger sample size of healthy horses. Also, it should be tested further by comparison of data from clinically ill horses to the reference quintiles of healthy horses.

Results of our study indicate that the use of proxies obtained from single basal plasma concentrations of glucose and, especially, insulin facilitates the assessment of insulin sensitivity and insulin responsiveness as well as the determination of compensatory insulin secretion and decompensation leading to hyperglycemia in horses. These aids should be useful in situations requiring multiple or repeated assessments that are unsuitable for complicated, expensive, specific quantitative methods, namely the euglycemic-hyperinsulinemic clamp technique and the minimal model, and inadequately described by nonspecific indications, such as fasting hyperinsulinemia. The use of proxies enables multiple determinations that are needed in population studies and in the initial characterization and subsequent monitoring of clinical cases. Proxies may be used with reference to quintiles and facilitated by graphs in which basal insulin concentration is entered to locate the quintile for insulin sensitivity and insulin and glucose concentrations are entered to locate the quintile for beta-cell response.

a. Case No. 02-44-68, Marion DuPont Scott Equine Medical Center, Leesburg, Va.

b. Beckman Instruments, Glucose Procedure No. 16-UV, Sigma Diagnostics, St Louis, Mo.

c. Coat-A-Count insulin, Diagnostic Products, Los Angeles, Calif.

Appendix

Calculation of proxies.

Proxies adapted for basal plasma glucose (mg/dL) and insulin (mU/L) concentrations

HOMA[25] = (glucose − insulin)/22.5
QUICKI[15] =(log [glucose × insulin])$^{-1}$
Glucose-to-insulin ratio = glucose/insulin
HOMA-BC%[25] = (20 × insulin)/(glucose − 63)
Insulin-to-glucose ratio = insulin/glucose
RISQI = 1/√insulin = insulin$^{-0.5}$
MIRG = (800 − 0.30 × [insulin − 50]^2)/(glucose − 30)

HOMA = Homeostasis model assessment. QUICKI = Quantitative insulin sensitivity check index. HOMA-BC% = Percent HOMA–beta-cell function. RISQI = Reciprocal of the square root of insulin. MIRG = Modified insulin-to-glucose ratio.

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