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Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats

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Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *Am J Physiol Endocrinol Metab* 295: E1269–E1276, 2008. First published September 15, 2008; doi:10.1152/ajpendo.90207.2008.—Insulin resistance plays a role in the pathogenesis of diabetes, including gestational diabetes. The glucose clamp is considered the gold standard for determining in vivo insulin sensitivity, both in human and in animal models. However, the clamp is laborious, time consuming and, in animals, requires anesthesia and collection of multiple blood samples. In human studies, a number of simple indexes, derived from fasting glucose and insulin levels, have been obtained and validated against the glucose clamp. However, these indexes have not been validated in rats and their accuracy in predicting altered insulin sensitivity remains to be established. In the present study, we have evaluated whether indirect estimates based on fasting glucose and insulin levels are valid predictors of insulin sensitivity in nonpregnant and 20-day-pregnant Wistar and Sprague-Dawley rats. We have analyzed the homeostasis model assessment of insulin resistance (HOMA-IR), the quantitative insulin sensitivity check index (QUICKI), and the fasting glucose-to-insulin ratio (FGIR) by comparing them with the insulin sensitivity (SI_{Clamp}) values obtained during the hyperinsulinemic-isoglycemic clamp. We have performed a calibration analysis to evaluate the ability of these indexes to accurately predict insulin sensitivity as determined by the reference glucose clamp. Finally, to assess the reliability of these indexes for the identification of animals with impaired insulin sensitivity, performance of the indexes was analyzed by receiver operating characteristic (ROC) curves in Wistar and Sprague-Dawley rats. We found that HOMA-IR, QUICKI, and FGIR correlated significantly with SI_{Clamp}, exhibited good sensitivity and specificity, accurately predicted SI_{Clamp}, and yielded lower insulin sensitivity in pregnant than in nonpregnant rats. Together, our data demonstrate that these indexes provide an easy and accurate measure of insulin sensitivity during pregnancy in the rat.

homeostasis model assessment of insulin resistance; quantitative insulin sensitivity check index; fasting glucose-to-insulin ratio; hyperinsulinemic isoglycemic clamp; calibration model

LATE PREGNANCY IS CHARACTERIZED BY THE DEVELOPMENT of insulin resistance both in humans (8, 9, 33) and rats (23, 30). Different procedures can be employed to assess insulin sensitivity, including the euglycemic-hyperinsulinemic clamp, the oral glucose tolerance test (OGTT), and various derivations of fasting glucose and insulin levels. The euglycemic-hyperinsulinemic clamp is considered the "gold standard" for measuring whole body insulin sensitivity in vivo because it directly measures the capacity of insulin to promote glucose utilization under steady-state conditions (12, 17). Although this method can provide a precise measure of the insulin sensitivity under physiological conditions, it is, however, a complicated and labor-intensive procedure. Therefore, simple but accurate estimates of insulin sensitivity are required to perform large-scale studies.

In the past, surrogate measures of insulin resistance have been developed based on measurements of fasting glucose and insulin concentrations (15, 21, 26). These indexes have been validated in humans by comparison with the hyperinsulinemic-euglycemic clamp, and they were found to correlate reasonably well with whole body insulin sensitivity determined with the clamp technique. The homeostasis model assessment of insulin resistance (HOMA-IR) was first described by Matthews et al. (26) as a measure of basal insulin sensitivity. The fasting glucose-to-insulin ratio (FGIR) has become popular since its first description as an accurate index of insulin sensitivity in women with polycystic ovary syndrome (21). The most recently proposed derivation, using simple fasting measures, is the quantitative insulin sensitivity check index (QUICKI), which is based on a log transform of the insulin glucose product (15). The log HOMA-IR and QUICKI are simply related by inversion and differ only by the normalizing constant used to calculate the HOMA-IR. To date, the best direct validation studies of simple surrogate indexes of insulin sensitivity, including HOMA-IR and QUICKI, were based on examining correlations with the reference glucose clamp method (15, 18, 25, 38). They provide a simple estimate for whole body insulin sensitivity with variability and discriminant power comparable to those of the euglycemic-hyperinsulinemic clamp (25), the minimal model (37), or the OGTT (16). Furthermore, QUICKI and logHOMA have been found to be excellent measures to predict the insulin sensitivity index obtained in the clamp (SI_{Clamp}) (10).

In animal studies, including different rat models, one or several of these indexes have been applied to quantify insulin sensitivity (19, 28, 34–36). Although a recent paper has analyzed the correlation between surrogate indexes of insulin resistance in mice (20), no study has been designed so far to validate these indexes in rats. This lack of information, therefore, violates the assumptions of the model (38). Furthermore, to our knowledge, no analysis of the discriminant power of these indexes has been performed in animals. The purpose of the present study was to evaluate whether the HOMA-IR, QUICKI, and FGIR indexes can be used to accurately estimate insulin sensitivity in nonpregnant and pregnant rats. We have performed this study in Wistar and Sprague-Dawley rats since they are the most commonly used strains for insulin-stimulated glucose measurements, and they are

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known to exhibit variations in whole body or tissular insulin sensitivity (22).

MATERIALS AND METHODS

Animals. The study includes a population of both female Wistar and Sprague-Dawley rats. Animals were housed at 22–24°C, with 12-h light cycles from 0800 to 2000 and free access to water and chow diet (Panlab, Barcelona, Spain). Animals were mated when they weighed 180–200 g. The beginning of pregnancy was determined by the presence of spermatozoids in vaginal smears, and animals were studied at *day 20* of pregnancy. Age- and sex-matched nonpregnant rats were studied in parallel. The experimental protocol was designed according to the recommendations of the Universities Federation for Animal Welfare Handbook on the Care and Management of Laboratory Animals and approved by the Animal Research Committee of the Faculty of Pharmacy, University CEU San Pablo, Madrid, Spain.

Hyperinsulinemic isoglycemic clamp studies. To assess insulin sensitivity, nonpregnant rats and rats at *day 20* of gestation were subjected to a hyperinsulinemic isoglycemic clamp after fasting for 6 h. In brief, blood samples were obtained from the tail tips for determination of basal glucose and insulin levels. Subsequently, animals were anesthetized with ketamine cocktail anesthesia (50 mg/ml ketamine, 5 mg/ml diazepam, and 1 mg/ml atropine; 5:4:1 vol/vol/vol at a final volume of 1 ml/ kg), and a Silastic brand catheter (0.02 in. ID, 0.037 in. OD; Dow Corning, Midland, MI) was placed in the right jugular vein and another catheter in the right femoral vein. Each catheter was connected to an infusion pump (Precidor Infusion Pump Type 5003; Infors HT, Denkendorf, Germany). Human insulin (Actrapid monocomponent; Novo, Copenhagen, Denmark) was infused by means of one pump at a constant rate of 16 μl/min (0.8 $IU \cdot h^{-1} \cdot kg$ body wt^{-1}) for 60 min. Glucose (20%) was infused at a variable rate through the second pump to maintain the blood glucose concentration constant at basal levels. Blood samples were collected from the tail tip at different time points to monitor the glycemia of the animals. Steady-state glucose infusion was generally achieved within 30 min after starting the clamp experiment. Additional blood samples (200 μl) were collected to determine the glucose and insulin concentration at the steady state in EDTA-plasma samples. The glucose disposal rate (M) was estimated as the rate of glucose infusion at the steady state normalized to body weight. SI_{Clamp}, as proposed by Ader and Bergman (1), was calculated as $M/(G \times \Delta I)$, where G is the steady-state blood glucose concentration, and ΔI is the increment of insulin concentration from basal levels to steady state.

Plasma analysis. Blood glucose during the clamp was measured by an immobilized glucose oxidase method (Reflolux IIM; Boehringer-Mannheim) (4). In EDTA-treated plasma samples, glucose was determined by an enzymatic colorimetric test (GOD-PAP; Roche Diagnostics, Barcelona, Spain) and insulin (standard curve range 0.1–10 μg/l; interassay: 8.5–9.4%; intra-assay: 1.4–4.6%; rat C-peptide not detectable) by a specific RIA kit for rats (Linco).

Calculation of insulin sensitivity indexes. From the short-term fasting plasma glucose (FPG) and insulin (FPI) values obtained in each animal before the clamp, the following indexes were calculated as estimates of insulin sensitivity: HOMA-IR, QUICKI, and FGIR.

HOMA-IR was calculated as the product of the FPG and FPI levels, divided by a constant, assuming that control young adult rats have an average HOMA-IR of 1, analogous to the assumptions applied in the development of HOMA-IR in humans (26). The equation was as follows HOMA-IR = (FPG × FPI)/2,430, where FPI was in microunits per milliliter and FPG in milligram per deciliter. QUICKI was calculated according to the original formula (15) as the inverse log sum of fasting insulin in microunit per milliliter and fasting glucose in milligram per deciliter. QUICKI = 1/[log(FPG) + log(FPI)]. Finally, FGIR was calculated as the ratio of FPG divided by FPI levels (21). FGIR = FPG/FPI, where FPG was in milligrams per deciliter and FPI in microunits per milliliter.

Calibration model analysis of surrogate insulin sensitivity indexes. To evaluate the ability of surrogate indexes to accurately predict insulin sensitivity as determined by the reference glucose clamp method, we used a calibration model to compare the ability of HOMA-IR, QUICKI, and FGIR to predict SI_{Clamp} as previously described by others (10, 20). In brief, calibration is the inverse of regression (7), thus using an estimated model $y = f(x;\theta)$, where x is the independent variable, y is the dependent variable, and θ is an unknown parameter, predicting a new y^* for a given x^* is regression. Conversely, predicting a new x^* for a given y^* is calibration. Accordingly, in the present study, we fitted a calibration model $x_i = \alpha + \beta y_i + \varepsilon_i$, where x_i is the SI_{Clamp}, y_i is each surrogate index, and ε_i is the random error for the ith subject. Even though SI_{Clamp} is measured with error, the assumption can be made that the measurement error of SI_{Clamp} (determined from a direct and data-intensive protocol) is very small relative to that of the indexes determined from single fasting measurements. Therefore, to simplify the analysis, we neglected the measurement error for SI_{Clamp} in our calibration model. For each surrogate index, two types of predicted residuals were considered. The first one is derived from the calibration model with all animals included and represents the difference between the measured SI_{Clamp} (x_i, for the i subject) and the fitted SI_{Clamp} for the same i subject. The second one is the residual obtained from a leave-one-out cross-validation model and represents the difference between the measured SI_{Clamp} (x_i, for the i subject) and the predicted SI_{Clamp} from the calibration model that excludes the i subject. Next, predictive accuracy was evaluated by root mean squared error of prediction (RMSE) and leave-one-out cross-validation type root mean squared error of prediction (CVPE). Smaller values of RMSE and CVPE indicate better prediction. The distribution of the obtained residuals for each index was displayed in box-and-whisker plots that ended at the first and third quartiles (27).

Data analysis and statistical evaluation. Results are expressed as means ± SE of 12–20 animals/group. Regarding the lognormal distribution of the insulin concentration, the statistical analyses were applied to the natural logarithm (log) of this parameter. All variables were evaluated for normality of distribution with the Kolmogorov-Smirnov goodness of fit. Where indicated in Tables 1–5 and the legends for Figs. 1–4, statistical comparisons between two groups were made with the Student's t-test. The level for statistical significance was set at 0.05 ($P < 0.05$). The relationship between insulin sensitivity, determined during the clamp SI_{Clamp} and indexes obtained from fasting glucose and insulin, was based on correlation analysis (Pearson coefficient) between pairs of indexes. Assessment of the performance of the various models was made using the receiver operating characteristic (ROC) curves by plotting the sensitivity against the corresponding false-positive rate (100-specificity) (14). The area under the ROC curve (AUC) was used as a measure of how well a continuous variable predicts the development of insulin resistance. A test with perfect discrimination power yields a ROC curve that passes through the upper left corner with an AUC of one (100% sensitivity and 100% specificity). Thus the closer the ROC area to one, the higher the discriminant power of the method. To construct the ROC curves, the presence of insulin resistance was defined according to the World Health Organization (European Group Insulin Resistance) (5) as a SI_{Clamp} value below the 25th percentile of the normal distribution in the nonpregnant animals (normal insulin sensitivity). To establish potential cutoff values for HOMA-IR, QUICKI, and FGIR, we determined the optimal decision point from the ROC curve, assigning equal weights to the sensitivity and specificity of the test. Statistical comparison of the areas under the ROC curves, derived from the same set of animals, was performed as described by Hanley and McNeil (13), taking into account the correlation between the areas that is induced by the paired nature of the data. Pearson correlation coefficients and ROC analysis were calculated using GraphPad programs (version 5.0 for Macintosh). Statistical comparisons of areas under ROC curves and Pearson correlation coefficients from the same

sample were made using the SimpleStat software. Calibration model and leave-one-out cross-validation analysis were performed by MATLAB version 7.

RESULTS

Changes of body weight and insulin sensitivity at late pregnancy. Table 1 shows the characteristics of the 67 Sprague-Dawley and Wistar rats included in the present study. Maternal body weight increased at late pregnancy in both strains of rats. Late-pregnant rats (*day 20*) had significantly lower plasma glucose levels in the presence of hyperinsulinemia. The values of glycemia were significantly higher in Sprague-Dawley than in Wistar rats ($P < 0.001$ for both nonpregnant and pregnant rats). SI_{Clamp} was used to obtain a direct measurement of insulin sensitivity. In addition, simple indexes of insulin resistance, namely HOMA-IR, QUICKI, and FGIR, were calculated from fasting glucose and insulin levels. As expected, insulin sensitivity measured during the clamp was significantly lower in the 20-day-pregnant than in the nonpregnant animals, both in the Wistar and Sprague-Dawley strain. As shown in Table 1, both the significantly higher HOMA-IR and the significantly lower QUICKI and FGIR indexes in the 20-day-pregnant animals compared with nonpregnant rats further confirmed the insulin resistant state associated with late pregnancy. Thus the rank order of insulin sensitivity determined by the fasting indexes corresponded to the correct rank order of insulin sensitivity determined by the SI_{Clamp}.

Validation studies of insulin sensitivity indexes. Because the direct measurement of insulin sensitivity with the glucose clamp is complex, we evaluated whether indirect estimates based on easy-to-measure fasting glucose and insulin levels can be used as valid predictors of insulin sensitivity in rats. For this purpose, first, we analyzed whether the indexes obtained from fasting glucose and insulin levels correlate with each other. Both in nonpregnant and late-pregnant Wistar rats, all indexes correlated significantly with each other. The analysis reveals a higher degree of correlation between QUICKI and HOMA-IR ($r = -0.970$ and -0.997, $P < 0.001$ for nonpregnant and pregnant rats, respectively) than between these latter indexes and FGIR ($r = -0.653$ and 0.781 for correlations of HOMA-IR to FGIR and of QUICKI to FGIR, respectively, in nonpregnant rats; $r = -0.558$ and 0.568 for correlations of HOMA-IR to FGIR and of QUICKI to FGIR, respectively, in late-pregnant rats). Correlation analysis including all Wistar animals shows the same pattern. Similar results were obtained with Sprague-Dawley rats, obtaining Pearson correlation coefficients between HOMA-IR and QUICKI close to 1 (-0.958 and -0.972 for nonpregnant and late-pregnant rats, respectively), and significantly lower ($P < 0.001$) between these indexes and FGIR ($r = -0.669$ and 0.676 for correlations of HOMA-IR to FGIR and of QUICKI to FGIR, respectively, in nonpregnant rats; $r = -0.705$ and 0.673 for correlations of HOMA-IR to FGIR and of QUICKI to FGIR, respectively, in late-pregnant rats). When the analysis was performed with the whole group of Sprague-Dawley animals (nonpregnant and late-pregnant rats), the results were very similar to those obtained with the Wistar strain.

The utility of these fasting indexes in estimating insulin resistance depends on the underlying correlation of these estimates with directly determined experimental data, being the euglycemic-hyperinsulinemic clamp the gold standard for quantifying insulin resistance. Table 2 shows the relationship between SI_{Clamp} and the HOMA-IR, QUICKI, and FGIR indexes, as estimated from plasma glucose and insulin levels, both in Wistar and Sprague-Dawley rats. First, the correlation analysis was performed separately with the nonpregnant and the late-pregnant group. The relationship (Pearson correlation coefficient) between SI_{Clamp} and the three indexes was statistically significant ($P < 0.05$ for all comparisons), independent of whether the rats were pregnant or not (Table 2). Because the slopes for the correlations of nonpregnant or pregnant rats in each strain were not significantly different, all data were pooled. When the entire group of animals, i.e., both nonpregnant and pregnant rats, was included in the analysis, the association of each of the indexes with SI_{Clamp} was even higher. QUICKI was the index that showed the best correlation to SI_{Clamp} in nonpregnant and late-pregnant rats in both strains of animals (nonpregnant: 0.745 and 0.869, late pregnant: 0.727 and 0.725, for Wistar and Sprague-Dawley rats, respectively; $P < 0.05$). In general, the weakest relationship was found for FGIR vs. SI_{Clamp} (nonpregnant: 0.497 and 0.614, late pregnant: 0.705 and 0.556, for Wistar and Sprague-Dawley rats, respec-

Table 1. *Effect of late pregnancy on body and adipose tissue weight and on biochemical parameters and insulin sensitivity indexes*

	Wistar Rats		Sprague-Dawley Rats	
	Nonpregnant	Late Pregnant (*day 20*)	Nonpregnant	Late Pregnant (*day 20*)
n	20	12	18	17
Body wt, g	211.0±4.4	308.0±9.2‡	221.0±7.1	326.0±9.2‡
Fasting glucose, mg/dl	87.6±2.6	65.9±1.1‡	126.0±3.2	91.0±1.8‡
Fasting insulin, μU/ml	30.80±1.95	64.50±1.90‡	38.10±2.24	69.20±6.07‡
SI_{Clamp}, $(10^{-4}\cdot dl\cdot min^{-1}\cdot kg^{-1})/(\mu U/ml)$	9.66±0.39	4.98±0.24‡	8.87±0.93	4.62±0.48‡
HOMA-IR	1.11±0.08	1.75±0.07‡	1.96±0.135	2.60±0.24*
QUICKI	0.294±0.003	0.276±0.001‡	0.274±0.003	0.266±0.002*
FGIR, mg/10^{-4} U	3.11±0.25	1.03±0.03‡	3.52±0.21	1.49±0.14‡

Data are mean values ± SE; *n*, no. of rats. SI_{Clamp}, insulin sensitivity index in the clamp; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; FGIR, fasting glucose-to-insulin ratio. Enzymatic colorimetric tests (GOD-PAP from Roche Diagnostics) were used to measure glucose in EDTA-plasma samples. Insulin was determined in plasma samples using a specific RIA kit for rats (Linco). Insulin sensitivity indexes, HOMA-IR, QUICKI, and FGIR, were calculated from short-term fasting plasma glucose and insulin values as described in MATERIALS AND METHODS. Values for plasma insulin were log transformed to equalize the variance between conditions. Comparisons between pregnant and nonpregnant rats from each strain were made by Student's *t*-test for unpaired data with equal or unequal variance as appropriate. *$P < 0.05$ and ‡$P < 0.001$, late-pregnant vs. nonpregnant rats.

Table 2. *Pearson correlation coefficients (r) between the insulin sensitivity index from isoglycemic hyperinsulinemic clamp (SI_{Clamp}) and insulin sensitivity indexes derived from fasting glucose and insulin*

	Wistar Rats		Sprague-Dawley Rats	
	r (95% CI)	*P*	*r* (95% CI)	*P*
Nonpregnant rats				
HOMA-IR	−0.714 (−0.879 to −0.398)	0.0004	−0.866 (−0.949 to −0.670)	<0.0001
QUICKI	0.745 (0.451–0.893)	0.0002	0.869 (0.677–0.950)	<0.0001
FGIR	0.497 (0.069–0.770)	0.026	0.614 (0.207–0.840)	0.0067
Late-pregnant rats				
HOMA	−0.685 (−0.904 to −0.183)	0.0139	−0.703 (−0.885 to −0.336)	0.0016
QUICKI	0.727 (0.262–0.918)	0.0074	0.725 (0.374–0.894)	0.0010
FGIR	0.705 (0.220–0.910)	0.0105	0.556 (0.102–0.818)	0.0205
All animals				
HOMA-IR	−0.850 (−0.924 to −0.712)	<0.0001	−0.718 (−0.848 to −0.506)	<0.0001
QUICKI	0.829 (0.676–0.914)	<0.0001	0.794 (0.627–0.891)	<0.0001
FGIR	0.813 (0.648–0.905)	<0.0001	0.750 (0.556–0.867)	<0.0001

CI, confidence interval.

tively; $P < 0.05$ compared with the correlation obtained between QUICKI and HOMA-IR vs. SI_{Clamp}).

Because the correlations of the three indexes with SI_{Clamp} were significant, independent of the gestational state or strain of the animals, and the slopes between these groups were not significantly different, all data were grouped. Figure 1 shows the correlation analysis, including both nonpregnant and late-pregnant Wistar and Sprague-Dawley rats, between SI_{Clamp} and HOMA-IR (*A*), QUICKI (*B*) or FGIR (*C*). Highly significant correlations ($P < 0.0001$) were obtained for all comparisons between each of the three indexes with SI_{Clamp}. As shown in Fig. 1*A*, the scatterplot of HOMA-IR was skewed hyperbolically, yielding data for late-pregnant Sprague-Dawley rats that were closer to the *y*-axis. However, log-transformed data yielded values that were virtually identical ($P = 0.24$) to those of HOMA-IR ($r = -0.704$, $P < 0.0001$ for HOMA-IR; $r = -0.736$, $P < 0.0001$ for logHOMA). Together, our results establish the usefulness of these indexes, QUICKI and HOMA-IR, for the quantification of insulin sensitivity in rats.

Correlation may be excellent even when prediction of reference values by the surrogate is poor. Thus it is important to evaluate the ability of surrogate indexes to accurately predict insulin sensitivity as determined by the reference glucose clamp method. In the present study, we used a calibration model to compare the ability of HOMA-IR, QUICKI, and FGIR to predict SI_{Clamp}. To this end, we regressed measured SI_{Clamp} for each animal on each surrogate index and fitted these data to a calibration model. Next, the predictions of SI_{Clamp} obtained from the calibration model (using the leave-one-out cross-validation approach) were plotted as a function of measured SI_{Clamp} by each index (Fig. 2). When a surrogate index perfectly predicts SI_{Clamp}, the values fall on a straight line with a slope of one and a y-intercept of zero. As shown in Fig. 2, the three indexes generated accurate predictions of SI_{Clamp} (with slopes of 1.02 ± 0.13, 1.02 ± 0.12, and 1.01 ± 0.12 ; intercepts of −0.19 ± 1.06, −0.10 ± 0.98, and −0.08 ± 0.95 for fitting between SI_{Clamp} vs. SI_{Clamp} predicted by HOMA-IR, QUICKI, and FGIR, respectively). In fact, statistical analysis indicates that the data did not differ significantly from a straight line. In addition, a linear least-squares fit between predicted SI_{Clamp} and measured SI_{Clamp} derived from the different indexes yielded correlation coefficients ($r = 0.692$, 0.720, and 0.728, for predictions by HOMA-IR, QUICKI, and FGIR, respectively, $P < 0.0001$ for all analysis) that did not significantly differ between each other.

Predictive accuracy was evaluated by RMSE of prediction. As shown in Table 3, when comparing RMSE and CVPE for each surrogate index, the obtained values were almost identical. To further evaluate the predictive accuracy of fasting indexes, the distribution of residuals was plotted in box-and-whiskers plots (data not shown). QUICKI was the index with the median of residuals closer to zero, and HOMA-IR was the only index with an outlier. Exclusion of this outlier did not significantly improve the analysis. Thus both in Wistar and Sprague-Dawley rats, the three surrogate indexes provide similar predictive accuracy for determining SI_{Clamp}.

We also wished to characterize the ability of these fasting indexes for the identification of rats with decreased insulin

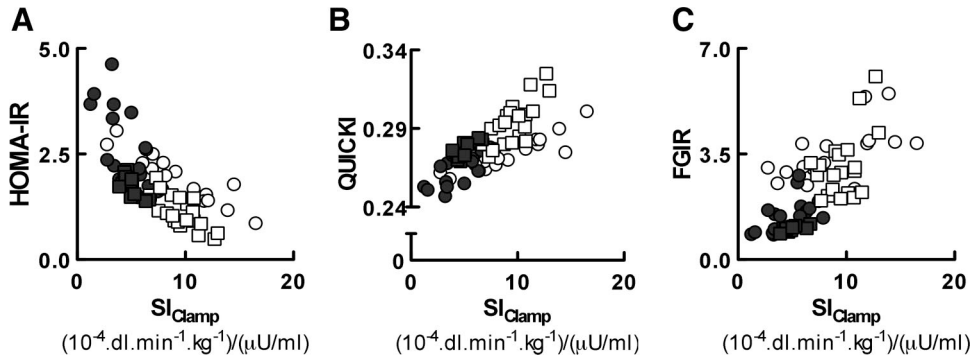

Fig. 1. Correlation between insulin sensitivity in the clamp (SI_{Clamp}) and indexes derived from fasting glucose and insulin concentrations. *A*: homeostasis model assessment of insulin resistance (HOMA-IR). *B*: quantitative insulin sensitivity check index (QUICKI). *C*: fasting glucose-to-insulin ratio (FGIR). Hyperinsulinemic isoglycemic clamp and index calculations were performed in each animal. Squares: Wistar rats; circles: Sprague-Dawley rats; open symbols: nonpregnant rats; filled symbols: late-pregnant rats. $P < 0.0001$ for all correlations.

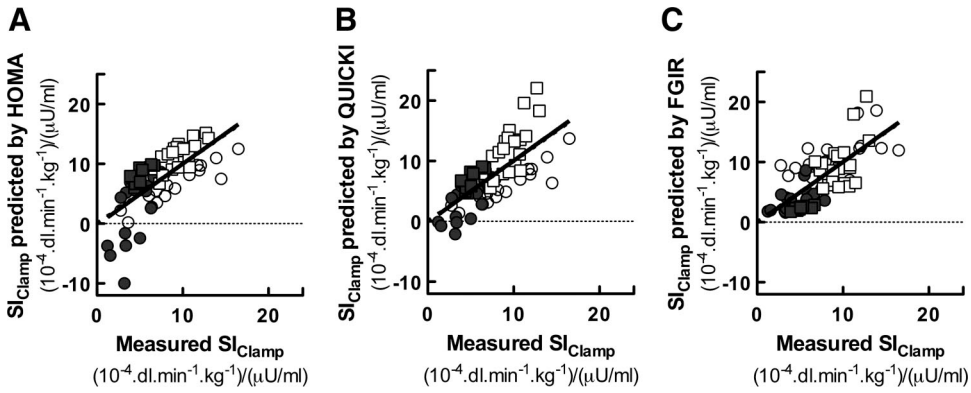

Fig. 2. Comparison between measured SI_{Clamp} and predicted SI_{Clamp} from various fasting-based indexes of insulin sensitivity in Wistar and Sprague-Dawley rats. Predicted SI_{Clamp} for each index was calculated by leave-one-out cross-validation analysis of the calibration model of SI_{Clamp} vs. each surrogate index as described in MATERIALS AND METHODS. *A*: results derived from HOMA-IR ($y = -0.19 + 1.02x$; $r = 0.692$). *B*: QUICKI ($y = -0.10 + 1.02x$; $r = 0.720$). *C*: FGIR ($y = -0.08 + 1.01x$; $r = 0.727$). The continuous line indicates the linear least-squares fit of predicted SI_{Clamp} vs. measured SI_{Clamp}. The dotted line indicates the ideal predictive accuracy (slope = 1 and intercept = 0).

sensitivity. For this purpose, we tested the performance of the different indexes against the SI_{Clamp} by ROC analysis. Insulin resistance was defined as the SI_{Clamp} value below the 25th percentile of the normal distribution (5). Because SI_{Clamp} was not significantly different between nonpregnant Wistar and Sprague-Dawley animals (data not shown), we combined the data obtained from both groups for the ROC analysis. As shown in Fig. 3, in the combined dataset obtained from adult nonpregnant Wistar and Sprague-Dawley rats, SI_{Clamp} follows a normal distribution. Thus insulin resistance was defined as $SI_{Clamp} < 7.1$ $(10^{-4}\cdot dl\cdot min^{-1}\cdot kg^{-1})/(\mu U/ml)$, and the ROC analysis was performed with insulin resistance yes/no as the outcome variable and with HOMA-IR, QUICKI, and FGIR as the test variables.

Figure 4 shows the ROC graphs where sensitivity is plotted against 100% specificity. The ideal test would yield 100% sensitivity and specificity, with the curve reaching the upper left corner of the graph. Therefore, the closer the ROC AUC to one, the greater is the overall accuracy of the test. Table 4 shows the AUC and 95% confidence intervals for the three indexes. In both Wistar and Sprague-Dawley rats, the AUC for the three indexes was statistically significant and very similar for both strains of rats. Thus because accuracy was similar in the different groups, ROC analysis was performed including all animals. As shown in Fig. 4, HOMA-IR (*A*), QUICKI (*B*), and FGIR (*C*) yielded ROC curves close to the left corner of the graph. The AUC for the three indexes was statistically significant ($P < 0.0001$), providing HOMA-IR and QUICKI with slightly better accuracy than the FGIR index (Table 4), although comparisons did not reach statistical difference. Furthermore, according to the cutoff value of SI_{Clamp} <7.1 $(10^{-4}\cdot dl\cdot min^{-1}\cdot kg^{-1})/(\mu U/ml)$ for defining insulin resistance, we examined the sensitivity and specificity of different cut-off values for HOMA-IR, QUICKI, and FGIR. The predictive performance of some cutoff values obtained for the investigated indexes is shown in Table 5. As indicated, the three indexes showed a similar performance with sensitivity values above 80% (84, 81, and 97% for HOMA-IR, QUICKI, and FGIR, respectively) and specificity values of 81% for HOMA-IR and QUICKI, and 70% for FGIR. Varying the cutoff value for SI_{Clamp} between 7.1 and 6.0 $(10^{-4}\cdot dl\cdot min^{-1}\cdot kg^{-1})/(\mu U/ml)$ did not change the pattern of the results. Furthermore, limiting ROC analysis to the nonpregnant rats yielded a similar performance for HOMA-IR and QUICKI (AUC of 0.9310 and 0.9406, respectively). Finally, when the ROC analysis was performed separately with independent SI_{Clamp} cutoff values for Wistar and Sprague-Dawley animals, a similar performance was obtained (data not shown). In conclusion, the studied fasting indexes, in particular QUICKI and HOMA-IR, provide highly sensitive and specific measurements of insulin sensitivity both in Wistar and Sprague-Dawley rats.

Table 3. *RMSE and CVPE calculated from calibration analysis of insulin sensitivity indexes derived from fasting glucose and insulin in Wistar and Sprague-Dawley rats*

	RMSE	CVPE
HOMA	3.327	3.512
QUICKI	3.096	3.241
FGIR	3.037	3.155

RMSE, square root mean error of prediction; CVPE, leave one out cross-validation root mean squared error of prediction. RMSE and CVPE were calculated from calibration analysis of HOMA-IR, QUICKI, and FGIR as described in MATERIALS AND METHODS.

DISCUSSION

Insulin resistance plays an important role in the pathogenesis of diabetes, including gestational diabetes. The euglycemic-hyperinsulinemic clamp is considered the gold standard for determining in vivo insulin sensitivity both in humans and in animal models. However, the glucose clamp is laborious, time consuming and, in the case of animal studies, implies anesthesia. Furthermore, the clamp is an invasive technique requiring

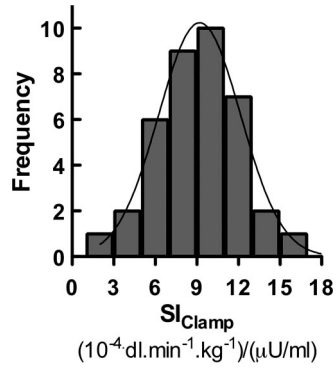

Fig. 3. Frequency histogram of insulin sensitivity (SI_{Clamp}) assessed by the isoglycemic-hyperinsulinemic clamp in nonpregnant Wistar and Sprague-Dawley rats.

Table 4. *Receiver operating characteristic (ROC) curves of simple indexes of insulin sensitivity in Wistar and Sprague-Dawley rats*

	HOMA-IR	QUICKI	FGIR
Wistar rats			
AUC	0.907	0.901	0.947
95% CI	0.804–1.009	0.794–1.008	0.846–1.049
P value	0.0001	0.0001	<0.0001
Sprague-Dawley rats			
AUC	0.877	0.855	0.855
95% CI	0.763–0.991	0.708–1.002	0.708–1.002
P value	0.0003	0.0007	0.0007
Both strains			
AUC	0.885	0.882	0.859
95% CI	0.808–0.963	0.803–0.960	0.773–0.945
P value	<0.0001	<0.0001	<0.0001

AUC, area under the curve.

Table 5. *Performance of indexes of insulin sensitivity in Wistar and Sprague-Dawley rats for the identification of insulin resistance according to the isoglycemic clamp technique*

	HOMA-IR	QUICKI	FGIR
Sensitivity, %	83.87	80.65	96.77
95% CI	66.27–94.55	62.53–92.55	83.30–99.92
Specificity, %	80.56	80.56	69.44
95% CI	63.98–91.81	63.98–91.81	51.89–83.65

Values shown are sensitivity and specificity for a cutoff value of HOMA-IR >1.716, QUICKI <0.2765, and FGIR <1.851.

the implantation of different catheters, which precludes its use in long-term studies. In humans, a number of simple indexes, derived from OGTT or from fasting glucose and insulin, have been obtained and validated against the glucose clamp, but these indexes have not been validated in rats. Because the rat is a widely used model to study insulin resistance, we have explored the usefulness of insulin indexes in Wistar and Sprague-Dawley rats. We have selected indexes based on biochemical parameters (insulin and glucose) that can be obtained in a single fasting blood sample. Some of these indexes, like the HOMA-IR, have been obtained from mathematical models performed in humans. In the HOMA-IR formula, a constant is applied to correct the value to the unit in normal subjects, assuming that they have an insulin resistance of one. For that reason, in the present study, we have adapted this index to the rat model. To evaluate the ability of HOMA-IR, QUICKI, and FGIR to predict insulin sensitivity as determined by the glucose clamp method, we performed a calibration model analysis. Finally, to assess the performance characteristics of these indexes to detect animals with impaired insulin sensitivity, we analyzed the predictive value of HOMA-IR, QUICKI, and FGIR for insulin resistance using ROC curves.

First, we performed a correlation study of theses indexes with SI_{Clamp}. The HOMA-IR index is based on the premise that circulating glucose and insulin levels are determined by a feedback loop between the liver and the pancreas; thus, this index essentially reflects on changes in hepatic insulin sensitivity. This model has been used for many years and has been validated in different physiological and pathological conditions in humans showing a very good correlation with the clamp method (6, 38). This index has also been used in some animal studies (19, 28, 34–36). However, to our knowledge, no validation with the clamp has been made in rats, and only two studies have been performed to validate HOMA-IR in animal models. The first study was conducted in cats, comparing glucose and insulin-based indexes with the minimal model. In this study, the authors found that the most useful predictors were basal plasma insulin and HOMA-IR (2). In a very recent report, performed in the mouse, it has been found that QUICKI and HOMA-IR were modestly correlated with SI_{Clamp}. The authors pointed out that this may be due to inherent technical difficulties in performing clamps in mice (20).

A criticism of the HOMA model is its deviation from linearity with increasing insulin resistance in human pathologies such as gestational diabetes (18) or type 2 diabetes (6). However, in our study performed with rats, similar correlations have been obtained for the overall insulin sensitivity derived from HOMA-IR or log HOMA-IR with the SI_{Clamp}, and these results are similar to those obtained in human pregnancy and in subjects with different degrees of insulin sensitivity (6). Therefore, despite its possible limitations in assessing peripheral insulin sensitivity, HOMA-IR is a good predictor of total body insulin sensitivity during pregnancy both in humans (18) and in rats (this study). In addition, our findings support the notion that HOMA-IR may be an useful tool to assess maternal insulin status independent of the rat strain used.

In the present study, insulin sensitivity assessed with the QUICKI index showed the strongest correlation with direct measurements of insulin sensitivity using the glucose clamp, being as significant as those reported previously in human studies (3, 15). The strength of the relation was maintained when we examined the data in nonpregnant and late-pregnant animals. Similar results have been observed in human pregnancy, where QUICKI has been found to be a good estimate of

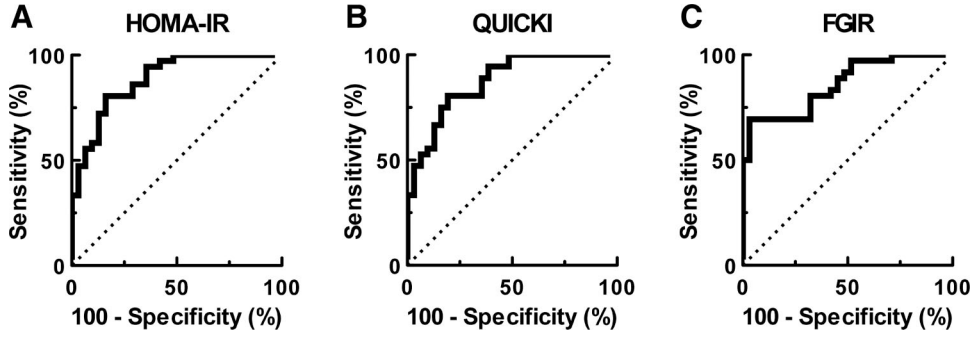

Fig. 4. Receiver operating characteristic (ROC) curves of different fasting-based indexes of insulin sensitivity, including both Wistar and Sprague-Dawley rats. For each index, sensitivity is plotted against 100% specificity. The ideal test should have sensitivity and specificity of 100% and reach the upper left corner of the graph. *A*: HOMA-IR. *B*: QUICKI. *C*: FGIR. ROC curve, continuous line; reference line, dotted line.

insulin sensitivity during both early and late pregnancy (18). Thus QUICKI provides an excellent alternative to the rather laborious and complex glucose clamps for assessing insulin sensitivity in rats. Although a comparison of insulin sensitivity between Sprague-Dawley and Wistar rats was beyond the scope of our study, it should be noted that the fasting indexes obtained here are in agreement with the previously reported lower insulin sensitivity in Sprague-Dawley compared with Wistar rats (31).

FGIR became a popular index (11) since its first application in women with polycystic ovary syndrome (21), but, as yet, this index has not been validated in animal models. In this study, although FGIR also correlated significantly with SI_{Clamp}, the correlation was weaker than the correlation found for HOMA-IR or QUICKI, both in nonpregnant and pregnant animals. In fact, it has been shown that FGIR is a conceptually flawed index of insulin sensitivity (29), since it does not appropriately reflect the physiology underlying insulin sensitivity, in particular when fasting glucose levels are not in the normal range. As shown above and according to the normal physiology of pregnancy, glucose is lower in late-pregnant rats than in the nonpregnant animals, which could artificially decrease the FGIR in the former group.

Thus HOMA-IR, QUICKI, and FGIR correlated significantly with each other, and, although each of these indexes correlated significantly with the SI_{Clamp}, statistical analysis of the correlation coefficients showed that the QUICKI provided the stronger correlations followed by HOMA-IR and FGIR.

To evaluate the predictive accuracy of these indexes, we used a calibration model, obtaining two criterion functions, the RMSE and a CVPE. CVPE is more robust than RMSE because it uses an estimate that excludes the *i*th subject when predicting results for the *i*th subject. CVPE also handles extreme data in a more rigorous way. In our study, both RMSE and CVPE were similar, suggesting that there were no extreme outliers that could have introduced a bias into the obtained results. Furthermore, we did not detect any differences of theses parameters comparing the different surrogate indexes. This suggests that, in rats, HOMA-IR, QUICKI, and FGIR provide similar accuracy in predicting SI_{Clamp}. Consistent with this finding, the distribution of residuals was very similar, showing only one outlier for HOMA-IR. In summary, this calibration model corroborates that, both in Wistar and Sprague-Dawley rats, the three indexes HOMA-IR, QUICKI, and FGIR provide comparable accuracy in predicting SI_{Clamp}.

Despite their use in animal models, the predictive performance of these indexes to identify insulin-resistant animals has not been examined so far. Therefore, we analyzed the performance of HOMA-IR, QUICKI, and FGIR against the SI_{Clamp} by ROC analysis, defining insulin resistance as an SI_{Clamp} value <7.1 $(10^{-14} \cdot dl \cdot min^{-1} \cdot kg^{-1})/(\mu U/ml)$ according to previously established criteria (5). Our data on the validity of these fasting indexes are robust and are based on data from two different rat strains and from animals with different degrees of insulin sensitivity (nonpregnant and late-pregnant rats). As evidenced by closely similar AUC values, we could not establish the superiority of any of the studied indexes of insulin sensitivity in Wistar or Sprague-Dawley rats. From the ROC analysis, different cutoff values and the corresponding values for sensitivity and specificity were obtained. Sensitivity was comparable for the three studied indexes, yielding the highest sensitivity for FGIR. This index, however, exhibited lower specificity when compared with HOMA-IR or QUICKI. HOMA-IR and QUICKI showed a comparable performance in nonpregnant and pregnant animals independent of whether they were Wistar or Sprague-Dawley rats.

All of these fasting indexes are highly dependent on glucose and fasting insulin levels. Although plasma glucose assays are very reproducible, insulin values have been reported to vary considerably between different laboratories (32). Although insulin assays have been improved during the last years, a proper standardization of insulin assays is still lacking (24). In addition, the variability of insulin measurements is further increased by the high biological variability of insulin levels, a consequence of its short serum half-life and pulsatile secretion. Thus, taking into account the lack of standardization of insulin assays, it is not possible to determine absolute and universal cutoff values that define insulin resistance by using an index that depends on insulin measurements. Despite these limitations, cutoff values for the surrogate indexes can serve as reference points in long-term studies provided that they are determined under identical conditions.

In conclusion, the present study shows that simple mathematical indexes derived from a single blood fasting sample, namely HOMA-IR, QUICKI, and FGIR, can provide an easy but accurate measure of insulin sensitivity in both nonpregnant and late-pregnant Wistar and Sprague-Dawley rats. Although not intended to replace the clamp, these fasting-based indexes, in particular QUICKI and HOMA-IR, offer important advantages in estimating insulin sensitivity. Because they are obtained from single fasting blood samples, they provide a useful tool for assessing insulin sensitivity in experimental settings in which the use of anesthesia is not recommended, such as pregnancy, as well as for long-term studies in which insulin resistance has to be assessed at different time points.

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