Comparison between surrogate indexes of insulin sensitivity and resistance and hyperinsulinemic euglycemic clamp estimates in mice

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1Diabetes Unit, National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, Maryland; 2Division of Biostatistics, Center for Devices and Radiological Health, United States Food and Drug Administration, Rockville, Maryland; 3Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

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Lee S, Muniyappa R, Yan X, Chen H, Yue LQ, Hong E-G, Kim JK, Quon MJ. Comparison between surrogate indexes of insulin sensitivity and resistance and hyperinsulinemic euglycemic clamp estimates in mice. Am J Physiol Endocrinol Metab 294: E261–E270, 2008. First published November 14, 2007; doi:10.1152/ajpendo.00676.2007.—Insulin resistance contributes to the pathophysiology of diabetes, obesity, and their cardiovascular complications. Mouse models of these human diseases are useful for gaining insight into pathophysiological mechanisms. The reference standard for measuring insulin sensitivity in both humans and animals is the euglycemic glucose clamp. Many studies have compared surrogate indexes of insulin sensitivity and resistance with glucose clamp estimates in humans. However, regulation of metabolic physiology in humans and rodents differs and comparisons between surrogate indexes and the glucose clamp have not been directly evaluated in rodents previously. Therefore, in the present study, we compared glucose clamp–derived measures of insulin sensitivity (GIR and SIClamp) with surrogate indexes, including quantitative insulin-sensitivity check index (QUICKI), homeostasis model assessment (HOMA), 1/HOMA, log(HOMA), and 1/ fasting insulin, using data from 87 mice with a wide range of insulin sensitivities. We evaluated simple linear correlations and performed calibration model; quantitative insulin-sensitivity check index

METHODS

Description of mouse models used. For the present study, we used glucose clamp data from a total of 87 mice with normal, decreased, or increased insulin sensitivity. Only data from mice where complete information was available to calculate both clamp–derived indexes of insulin sensitivity and surrogate indexes of insulin sensitivity were used. Consequently, data from 23 out of an original cohort of 110 mice were not included in this study for final analyses. C57BL/6 mice with normal insulin sensitivity (n = 10) were used as controls for insulin-resistant mice on a high-fat diet for 3 wk (n = 10), 6 wk (n = 6), or 3 mo (n = 10) (24). Wild-type FVB mice with normal insulin sensitivity (n = 6)
were littersate controls for insulin-resistant transgenic mice overexpressing the protein-tyrosine phosphatases PTP1B or LAR in skeletal muscle (MCK-hPTP1B, n = 7; MCK-hLAR, n = 7; Ref. 36). Wild-type C57BL/6 × FVB mice with normal insulin sensitivity (n = 4) were littersate controls for insulin-resistant transgenic mice overexpressing a dominant-negative mutant of CEACAM1 in liver (L-SACC1, n = 4; Ref. 25). Wild-type C57BL/6 mice with normal insulin sensitivity (n = 4) were littersate controls for Par-1b null mice with increased insulin sensitivity (Par-1b KO, n = 3; Ref. 16). Wild-type C57BL/6J mice with normal insulin sensitivity (n = 5) were littersate controls for mice with functionally deficient SHP-1 protein that have increased insulin sensitivity (Ptpn6me-v/vme-v, n = 6; Ref. 10). Wild-type C57BL/6J mice with normal insulin sensitivity (n = 3) were used as controls for CASA/Rk mice with increased insulin sensitivity (n = 2; Ref. 33).

Hyperinsulinemic euglycemic clamp. Glucose clamp procedures in conscious unanesthetized mice were performed as described previously (10, 16, 24, 25, 36). In brief, mice were fasted overnight (~15 h) and a 2-h hyperinsulinemic euglycemic clamp procedure was conducted with a primed-continuous infusion of insulin (15 pmol.kg⁻¹.min⁻¹; humulin; Eli Lilly, Indianapolis, IN) and a variable infusion of 20% glucose to maintain euglycemia. Basal and insulin-stimulated whole-body glucose turnover was estimated with a continuous infusion of [3-H]glucose (PerkinElmer Life and Analytical Sciences, Boston, MA) for 2 h before (0.05 μCi/min) and throughout the clamps (0.1 μCi/min), respectively. The glucose infusion rate (GIR) adjusted for body weight was used as one measure of insulin sensitivity. In addition, we calculated a clamp-derived index of insulin sensitivity as SIClamp = M/(G × ΔI) adjusted for body weight where M is the steady-state glucose infusion rate (mg/min), G is the steady-state blood glucose concentration (mg/dl), and ΔI is the difference between basal and steady-state plasma insulin concentrations (μU/ml). Rates of basal hepatic glucose production (HGP) and insulin-stimulated whole-body glucose turnover were determined as the ratio of the [1H]glucose infusion rate (disintegrations/min) to the specific activity of plasma glucose (dpm/μmol) at the end of the basal period and during the final 30 min of the clamp procedure, respectively. HGP during hyperinsulinemic clamp conditions was determined by subtracting the GIR from whole-body glucose turnover. All procedures were approved by the appropriate Animal Care and Use Committees.

Surrogate indexes of insulin sensitivity and resistance. Surrogate indexes were calculated from fasting blood glucose and plasma insulin concentrations as follows: QUICKI = 1/(log(I₀) + log(G₀)), where I₀ is fasting insulin (μU/ml) and G₀ is fasting glucose (mg/dl; Ref. 17); and HOMA = (G₀ × I₀)/22.5 (with glucose expressed as mmol/l and insulin expressed as μU/ml; Ref. 21). Other surrogate indexes examined were 1/HOMA, log(HOMA) and 1/fasting insulin.

Calibration model analysis of surrogate indexes adjusted for body weight. Calibration is inverse regression (4). For the model y = f(x; θ) + ε, x is the explanatory variable, y is the response variable, θ is an unknown parameter, and ε is the random error. Using an estimated model y = f(x; β) to predict a new y* for a given x* is regression. Conversely, predicting a new x* for a given y* is calibration. If the value of x is prespecified as part of an experimental design, this is called classical or controlled calibration. Since QUICKI (or other surrogates), GIR (or SIClamp), and weight are measured with error from an experimental population, random calibration is the more appropriate method to use. In random calibration, there is no difficulty in specifying the conditional distribution of x given y, so that random calibration is similar to regression in prediction. Here we fitted a calibration model xᵢ = α + β₁Sᵢ + β₂Wᵢ + β₃SᵢWᵢ + εᵢ, where xᵢ is GIR (or SIClamp), Sᵢ is the surrogate index, Wᵢ is the body weight, SᵢWᵢ is the multiplication of surrogate index and body weight, and εᵢ is the random error for the i th subject. It was assumed that the random error had Gaussian distribution with mean of 0 and a constant variance. Even though GIR (or SIClamp) was measured with error, it was assumed for our model that the measurement error of GIR (or SIClamp) was determined from a robust, direct, and data-intensive protocol) was very small relative to measurement error of simple surrogates determined from single fasting measurements (e.g., QUICKI) and measurement error of body weight. Therefore, to simplify the analysis, we neglected the measurement error for GIR (or SIClamp) in our calibration model.

For each surrogate index, two types of predicted residuals were considered. The first type of residual is the difference between measured GIR (or SIClamp) (xᵢ for the ith subject) and fitted GIR (or SIClamp), δᵢ = xᵢ − x̂ᵢ, where xᵢ is derived from the calibration model with all subjects included in the estimation of model parameters α, β₁, β₂ and β₃. The second type of residual considered is a cross-validation type predicted residual εᵢ = xᵢ − x̂ᵢ, where xᵢ is still measured GIR (or SIClamp), but x̂ᵢ is predicted SIClamp from the calibration model that excludes the ith subject. The subscript (i) means “with the ith subject deleted.” From these two types of residuals, criterion functions were used to evaluate prediction accuracy: square root of the mean squared error of prediction: RMSE = [Σeᵢ²/n]¹/₂ and leave-one-out cross-validation-type root mean squared error of prediction: CVPE = [Σeᵢ²/n]¹/₂. Smaller values of RMSE and CVPE indicate better predictive power. RMSE is likely to underestimate prediction errors while CVPE is more robust.

Statistical analysis. Pearson correlation coefficients (R) and respective P values were calculated to assess the statistical significance of the model using a linear least-squares fit method to obtain the linear regression. To compare predictive accuracy of QUICKI (adjusted for weight) and other surrogates (adjusted for weight) in terms of CVPE and RMSE, we performed hypothesis testing with the one-sided alternative hypothesis that weight-adjusted QUICKI had a smaller RMSE or CVPE than another weight-adjusted surrogate using a Bootstrap percentile method with 60,000 replications performed for each comparison (11). The bootstrap method is appropriate because when P values calculated from comparison of RMSE and CVPE were for pair-wise comparisons. For example, when weight-adjusted QUICKI and weight-adjusted HOMA were compared with respect to CVPE based on 87 mice, a bootstrap percentile method with 60,000 replications was used to get a sample of 60,000 differences in CVPE [CVPE (HOMA) – CVPE (QUICKI)], and then a P value for one-sided superiority testing was estimated as the proportion of the bootstrap replications less than zero. One-sided hypothesis testing was used because multiple previous studies in humans have demonstrated the superiority of QUICKI as a surrogate index of insulin sensitivity from a variety of perspectives supporting an a priori expectation. P < 0.05 was considered to indicate statistical significance. The software used for statistical analysis and the random calibration model was SAS System V9.

RESULTS

Mice. Our original cohort consisted of 110 mice. However, to do complete analyses for both GIR and SIClamp, 23 out of these 110 mice were excluded because of incomplete data sets. Metabolic characteristics of the 87 mice analyzed in our study under fasting and steady-state glucose clamp conditions are shown in Table 1. Note that appropriate control mice corresponding to each experimental group have been combined together for a total of 32 control mice. Groups of mice with decreased insulin sensitivity include MCK-hPTP1B transgenic mice (36), MCK-hLAR transgenic mice (36), L-SACC1 transgenic mice (25), and mice fed with high-fat diet for 3 wk, 6 wk, or 3 mo, respectively (24). Groups of mice with increased insulin sensitivity include Par-1b null mice (16), Ptnpho-v/vme-v mice (10), and CASA/Rk mice (33). Determinations of insulin sensitivity and resistance. We used both the steady-state GIR as well as SIClamp to obtain
direct estimates of insulin sensitivity adjusted for body weight from glucose clamp data (Table 2). In addition, from fasting insulin and glucose data, we calculated a variety of simple surrogate indexes of insulin sensitivity and resistance, including QUICKI, HOMA, 1/HOMA, log(\text{HOMA}), and 1/fasting insulin (Table 2). As expected, measures of insulin sensitivity derived from glucose clamp data demonstrated lower mean GIR and \text{SI}_{\text{clamp}} for groups of mice with decreased insulin sensitivity and higher mean GIR and \text{SI}_{\text{clamp}} for groups of mice with increased insulin sensitivity when compared with mean values from the control group with normal insulin sensitivity. Similarly, for surrogate indexes of insulin sensitivity (QUICKI, 1/HOMA, and 1/fasting insulin), lower mean values were observed for groups of mice with decreased insulin sensitivity while higher mean values were observed for groups of mice with increased insulin sensitivity when compared with mean values from the control group with normal insulin sensitivity. Finally, for surrogate indexes of insulin resistance [HOMA and log(\text{HOMA})], higher mean values were observed for groups of mice with decreased insulin sensitivity while lower mean values were observed for groups of mice with increased insulin sensitivity when compared with mean values from the control group with normal insulin sensitivity. Thus, the rank order of insulin sensitivity and resistance determined by all of the surrogate indexes tested corresponded to the correct rank order of insulin sensitivity determined by direct measures obtained from glucose clamp data. Subgroup analyses in specific groups of mice were not considered a priori due to the small sample sizes of subgroups.

**Correlations between clamp-derived measures of insulin sensitivity and surrogate indexes of insulin sensitivity and resistance.** To determine the relative merits of various surrogate indexes of insulin sensitivity and resistance, we first analyzed linear correlations between all of the surrogate indexes and GIR (Fig. 1) or \text{SI}_{\text{clamp}} (Fig. 2). Modest linear correlations were observed for all comparisons. 1/fasting insulin demonstrated the weakest correlation with GIR or \text{SI}_{\text{clamp}} (R ≈ 0.30; P < 0.008). All of the other surrogate indexes [QUICKI, HOMA, 1/HOMA, and log(\text{HOMA})] had stronger linear correlations with GIR or \text{SI}_{\text{clamp}} (R ≈ 0.45; P < 0.0001). In the original cohort (n = 110), linear correlations between GIR and other surrogate indexes [QUICKI, HOMA, 1/HOMA, and log(\text{HOMA})] were also comparable (R ≈ 0.40; P < 0.0001). However, based on the similar correlation coefficients, we could not distinguish any particular advantage among these remaining surrogate indexes. Moreover, the linear correlations between surrogate indexes and clamp-derived estimates of insulin sensitivity we observed in mice were not as substantial as those previously reported in humans [5, 6, 17, 19]. In addition, the linear correlations between surrogate indexes and whole-body glucose turnover were generally weaker than correlations with GIR or \text{SI}_{\text{clamp}} (data not shown). Finally, we did not observe any substantial linear correlations between any of the surrogate indexes tested and insulin-mediated suppression of HGP during the glucose clamp (data not shown).

**Correlations between GIR or \text{SI}_{\text{clamp}} and body weight in mice.** One potential explanation for disparity between mice and humans with respect to linear correlations of direct and surro-

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**Table 1.** Metabolic characteristics of mice grouped according to insulin sensitivity relative to respective appropriate control mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>\text{Control}</th>
<th>hPTP1B-TG</th>
<th>hLAR-TG</th>
<th>L-SACC1</th>
<th>3-wk HFD</th>
<th>6-wk HFD</th>
<th>3-mo HFD</th>
<th>Par-1bKO</th>
<th>Ptpn-6me/me-v</th>
<th>CASA/Rk</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>32</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26 ± 1</td>
<td>33 ± 1</td>
<td>34 ± 1</td>
<td>27 ± 2</td>
<td>30 ± 1</td>
<td>28 ± 1</td>
<td>38 ± 1</td>
<td>18 ± 1</td>
<td>14 ± 2</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>131 ± 6</td>
<td>147 ± 13</td>
<td>168 ± 16</td>
<td>135 ± 7</td>
<td>175 ± 13</td>
<td>165 ± 6</td>
<td>142 ± 9</td>
<td>97 ± 5</td>
<td>72 ± 14</td>
<td>150 ± 2</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>14 ± 1</td>
<td>29 ± 5</td>
<td>23 ± 2</td>
<td>15 ± 2</td>
<td>17 ± 1</td>
<td>20 ± 6</td>
<td>38 ± 6</td>
<td>9 ± 2</td>
<td>11 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Clamp insulin, μU/ml</td>
<td>109 ± 4</td>
<td>113 ± 7</td>
<td>115 ± 9</td>
<td>105 ± 5</td>
<td>103 ± 7</td>
<td>94 ± 3</td>
<td>125 ± 7</td>
<td>121 ± 4</td>
<td>113 ± 6</td>
<td>110 ± 7</td>
</tr>
<tr>
<td>Clamp insulin, μU/ml</td>
<td>53 ± 4</td>
<td>76 ± 7</td>
<td>74 ± 8</td>
<td>65 ± 10</td>
<td>42 ± 4</td>
<td>38 ± 4</td>
<td>62 ± 5</td>
<td>30 ± 2</td>
<td>39 ± 4</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>GIR, mg·kg⁻¹·min⁻¹</td>
<td>43 ± 2</td>
<td>25 ± 5</td>
<td>26 ± 4</td>
<td>43 ± 3</td>
<td>18 ± 6</td>
<td>15 ± 4</td>
<td>12 ± 1</td>
<td>61 ± 5</td>
<td>52 ± 5</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>Basal HGP, mg·kg⁻¹·min⁻¹</td>
<td>16 ± 1</td>
<td>15 ± 1</td>
<td>18 ± 2</td>
<td>20 ± 2</td>
<td>17 ± 1</td>
<td>18 ± 2</td>
<td>10 ± 1</td>
<td>15 ± 2</td>
<td>17 ± 2</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Clamp HGP, mg·kg⁻¹·min⁻¹</td>
<td>0 ± 1</td>
<td>7 ± 4</td>
<td>7 ± 3</td>
<td>10 ± 2</td>
<td>9 ± 3</td>
<td>14 ± 3</td>
<td>8 ± 1</td>
<td>0 ± 5</td>
<td>−4 ± 3</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>WBGt, mg·kg⁻¹·min⁻¹</td>
<td>43 ± 2</td>
<td>32 ± 3</td>
<td>33 ± 2</td>
<td>52 ± 1</td>
<td>28 ± 2</td>
<td>29 ± 2</td>
<td>21 ± 1</td>
<td>61 ± 8</td>
<td>49 ± 2</td>
<td>47 ± 3</td>
</tr>
</tbody>
</table>

Data shown are mean ± SE from glucose clamp studies at baseline (fasting) or under steady-state clamp conditions. TG, transgenic mouse; KO, knockout mouse; GIR, glucose infusion rate; HGP, hepatic glucose production; \text{SI}_{\text{clamp}}, whole body glucose turnover; HFD, high fat diet.

**Table 2.** Glucose clamp derived measures of insulin sensitivity (GIR and \text{SI}_{\text{clamp}}) as well as surrogate indexes of insulin sensitivity and resistance in mice grouped according to insulin sensitivity

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>GIR, mg·kg⁻¹·min⁻¹</th>
<th>\text{SI}_{\text{clamp}}, 10⁻⁴·dU/kg⁻¹·min⁻¹</th>
<th>QUICKI</th>
<th>HOMA</th>
<th>1/HOMA</th>
<th>Log(\text{HOMA})</th>
<th>1/Fasting Insulin, ml/μU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32</td>
<td>43.5 ± 2.0</td>
<td>131 ± 12</td>
<td>0.315 ± 0.004</td>
<td>4.51 ± 0.44</td>
<td>0.30 ± 0.03</td>
<td>0.590 ± 0.043</td>
<td>0.092 ± 0.010</td>
</tr>
<tr>
<td>Decreased insulin sensitivity</td>
<td>44</td>
<td>21.1 ± 2.3</td>
<td>68 ± 10</td>
<td>0.287 ± 0.002</td>
<td>8.58 ± 0.61</td>
<td>0.14 ± 0.01</td>
<td>0.892 ± 0.029</td>
<td>0.053 ± 0.004</td>
</tr>
<tr>
<td>Increased insulin sensitivity</td>
<td>11</td>
<td>51.1 ± 3.0</td>
<td>195 ± 18</td>
<td>0.345 ± 0.008</td>
<td>2.36 ± 0.46</td>
<td>0.54 ± 0.07</td>
<td>0.310 ± 0.069</td>
<td>0.113 ± 0.014</td>
</tr>
</tbody>
</table>

Data shown are mean ± SE from glucose clamp studies at baseline (fasting) or under steady-state clamp conditions. GIR, glucose infusion rate; QUICKI, quantitative insulin sensitivity check index; HOMA, homeostasis model assessment.

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gate indexes of insulin sensitivity may be that the ratio of body surface area to mass is quite different. In addition, unlike humans, rodents do not have a true physiological fasting state and their body weight may change significantly upon fasting. Therefore, we examined linear correlations between GIR or SIClamp and body weight per se in our mice (Fig. 3). Body weight of our mice had a substantially stronger correlation with clamp-derived measures of insulin sensitivity than any of the surrogate indexes tested ($R = 0.65$; $P < 0.0001$ for GIR).

Therefore, it may be useful in mice to adjust surrogate indexes of insulin sensitivity and resistance according to body weight.

Calibration model analysis of surrogate indexes adjusted for body weight.

We tested the absolute accuracy of various surrogate indexes adjusted for body weight using calibration model analysis. The calibration model generates predictions of GIR (Fig. 4) or $SI_{\text{Clamp}}$ (Fig. 5) based on data from each surrogate index. If a surrogate index adjusted for body weight perfectly predicts GIR or $SI_{\text{Clamp}}$, a plot of the predicted vs. observed values of GIR or $SI_{\text{Clamp}}$ for a given surrogate index would generate a straight line with a slope of 1 and a y-intercept of 0. To generate the plots in Figs. 4 and 5, we used the leave-one-out cross-validation approach to the calibration model (as described in MATERIALS AND METHODS). When comparing error functions CVPE or RMSE for each of the surrogate indexes, the error was much smaller for predicting GIR than for predicting $SI_{\text{Clamp}}$ (Table 3). However, when evaluating the absolute accuracy of all of the surrogate indexes adjusted for body weight that were tested, no significant differences were noted among any of the indexes when compared with QUICKI (evaluated by either CVPE or RMSE and

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**Fig. 1.** Linear correlation between glucose infusion rate (GIR) and various surrogate indexes of insulin sensitivity and resistance derived from glucose clamp studies; $n = 87$ with 32 control mice ($\bullet$), 44 mice from decreased insulin sensitivity groups ($\circ$), and 11 mice from increased insulin sensitivity groups ($\triangle$). Solid line represents the linear least squares fit between GIR and each surrogate index of insulin sensitivity and resistance for all mice. Correlation coefficients ($R$) and respective $P$ values are shown in A–E. A: results derived from Quantitative insulin-sensitivity check index (QUICKI). B: results derived from homeostasis model assessment (HOMA). C: results derived from $1/$HOMA. D: results derived from $\log($HOMA$)$. E: results derived from $1/$fasting insulin.
either with GIR or SI\textsubscript{clamp}; Table 3). Thus in mice, adjusting any of the common surrogate indexes of insulin sensitivity and resistance for body weight results in reasonable predictive accuracy for determining GIR.

**DISCUSSION**

Insulin resistance plays a significant role in the pathogenesis of diabetes and is strongly associated with obesity and cardiovascular diseases (9, 28). Mouse models of these human diseases with acquired or genetic forms of insulin resistance and sensitivity are useful for gaining insight into pathophysiological mechanisms, for evaluating therapeutic interventions, and for screening novel therapeutic compounds and approaches. The hyperinsulinemic euglycemic clamp technique is considered the reference standard for determining insulin sensitivity and resistance in both humans and rodents. However, the glucose clamp is laborious, time consuming, and technically demanding to perform (more so in rodents than in humans). In humans, a number of surrogate indexes for insulin sensitivity and resistance have been developed that correlate reasonably well with glucose clamp results (2, 3, 5, 17, 20, 21, 23). However, the present study is the first to evaluate comparisons between surrogate indexes of insulin sensitivity and resistance and glucose clamp estimates in mice. It is important to explicitly evaluate these comparisons in mice because validation of simple, accurate, and reliable surrogate indexes of insulin sensitivity and resistance in mice will be valuable for drug development and screening purposes as well as for other studies where feasibility issues prevent the use of the glucose clamp.

**Comparisons between glucose clamp estimates and surrogate indexes of insulin sensitivity and resistance.** In the present study, we used data from a total of 87 mice that exhibited a
To more rigorously evaluate the surrogate indexes we also evaluated linear correlations between surrogates and either GIR or SIClamp. All of the surrogate indexes evaluated [QUICKI, HOMA, 1/HOMA, log(HOMA), and 1/fasting insulin] had modest linear correlations with both GIR and SIClamp. However, the correlation between GIR or SIClamp and 1/fasting insulin ($R \approx 0.30$) was not as high as with the other surrogates ($R \approx 0.45$). Thus, this analysis revealed modest linear correlations between direct measures of insulin sensitivity and surrogate indexes in mice but no clear advantage among QUICKI, HOMA, 1/HOMA, or log(HOMA). It is possible that using larger numbers of mice may improve correlations between surrogates and glucose clamp-derived measures of insulin sensitivity. However, this seems unlikely as linear correlations between GIR and surrogates for 110 mice were similar to those we observed for the set of 87 mice with complete data. By contrast, in humans, both QUICKI and log(HOMA) have much more substantial and stronger linear correlations with glucose clamp estimates in multiple independent studies ($R \approx 0.70 – 0.90$; Refs. 5, 6, 17, 19, 29, 34, 35).

There are several possible explanations for these differences observed between mice and humans. First, all of the surrogate indexes rely on the assumption that fasting glucose and insulin levels represent a basal steady-state condition. However, unlike humans, rodents have no true physiological fasting state. Mice feed primarily during the dark hours and prolonged fasting (15 h) increases peripheral insulin sensitivity and decreases lean body mass, hepatic glycogen content, plasma glucose, and insulin levels (1, 12). Consequently, changes in peripheral insulin sensitivity, fasting insulin, and glucose concentrations during fasting in mice occur in a time-dependent fashion. That is, nonphysiological fasting conditions imposed on mice may not determine steady-state conditions. Second, the ratio of body surface area to mass in mice is quite different than in humans. Thus, mice may have substantial changes in body weight upon fasting (1). Indeed, we found a more substantial linear correlation between GIR (or SIClamp) and body weight per se than with any of the surrogate indexes tested. This suggests that in mice it may be useful to adjust surrogate indexes for body weight. Third, it is likely that the glucose clamp technique in mice may not be as accurate and reliable as in humans (1). Mice have very small blood volumes (~2 ml) so that limited sampling ability makes it difficult to achieve and rigorously verify steady-state clamp conditions (especially with respect to insulin levels). For example, in humans, the clamp steady-state values for GIR, blood glucose, and plasma insulin typically vary by <5% over 60 min. In mice, because of small blood volumes, it is not possible to measure multiple insulin concentrations during the clamp to verify steady-state insulin levels. In addition, the glucose clamp procedure is stressful in mice (even if done under anesthetized conditions). Inherent limitations and variability associated with measurement of low levels of plasma insulin during the fasting state may also contribute to the modest correlations observed in the present study. Another consideration is that many monogenic forms of insulin resistance in mice preferentially cause skeletal muscle insulin resistance without substantially affecting hepatic insulin sensitivity. The GIR from the clamp predominantly reflects skeletal muscle insulin sensitivity, while the surrogate indexes based on fasting glucose and insulin levels primarily reflect hepatic insulin sensitivity. In
humans, under most conditions, hepatic and muscle insulin sensitivity and resistance are proportional. However, in mice, if skeletal muscle and hepatic insulin sensitivity and resistance are uncoupled, this may contribute to weaker correlations between surrogate indexes and glucose clamp estimates. In rats, correlations between surrogate measures of insulin sensitivity (QUICKI, HOMA, and fasting insulin) and clamp-derived estimates of glucose disposal are also modest (31). It is possible that limitations that apply to mice may also affect similar studies in rats.

**Calibration model analysis of surrogate indexes adjusted for body weight.** To evaluate the absolute accuracy of various surrogate indexes of insulin sensitivity and resistance, we used calibration model analysis to assess the predictive accuracy of each surrogate index adjusted for body weight. For this analysis, we assumed that error in GIR or $S_{IClamp}$ is small when compared with error in surrogate indexes. With the use of calibration model analysis, weight-adjusted QUICKI (or other weight-adjusted surrogate indexes) was reasonable at predicting GIR. In contrast, in humans, QUICKI and log(HOMA) were both excellent at predicting $S_{IClamp}$ with a much smaller CVPE and RMSE compared with this study in mice (5). In addition, both error functions CVPE and RMSE were consistently smaller for surrogates to predict GIR than for surrogates to predict $S_{IClamp}$. This suggests that $S_{IClamp}$ in mice may have a larger error than GIR for reasons related to small blood volumes and difficulties in obtaining sufficient insulin determinations in mice as described above. In humans, QUICKI and log(HOMA) have the best linear correlation with $S_{IClamp}$ estimates of insulin sensitivity and resistance (5). However, in our calibration model analysis of mice, there were no significant differences in predictive accuracy among any of the surrogates tested. Moreover, predictive accuracy of surrogate indexes in mice even

Fig. 4. Comparison between observed GIR during glucose clamp and predicted GIR derived from the calibration model of various surrogate indexes of insulin sensitivity and resistance adjusted for body weight (leave-one-out cross validation analysis) as described in MATERIALS AND METHODS; $n = 87$ with 32 control mice ($\bullet$), 44 mice from decreased insulin sensitivity groups ($\odot$), and 11 mice from increased insulin sensitivity groups ($\triangle$). The dashed line indicates ideal predictive accuracy with a slope of 1 and intercept of 0. A: results derived from QUICKI adjusted for body weight. B: results derived from HOMA adjusted for body weight. C: results derived from $1/HOMA$ adjusted for body weight. D: results derived from log(HOMA) adjusted for body weight. E: results derived from $1/fasting$ insulin adjusted for body weight.
after adjusting for body weight as well as linear correlations is not substantial as in humans. This may reflect intrinsic differences between human and rodent physiology (e.g., using monogenic mouse models of polygenic human disease) as well as increased technical difficulties in performing glucose clamps in mice with small blood volumes.

In summary, in mice, many common simple surrogate indexes of insulin sensitivity and resistance are modestly corre-

Table 3. CVPE and RMSE calculated from calibration analysis of various surrogate indexes of insulin sensitivity adjusted for weight

<table>
<thead>
<tr>
<th></th>
<th>GIR</th>
<th></th>
<th></th>
<th>SI_{clamp}</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CVPE</td>
<td>P</td>
<td>RMSE</td>
<td>CVPE</td>
<td>P</td>
<td>RMSE</td>
</tr>
<tr>
<td>QUICKI</td>
<td>13.94</td>
<td></td>
<td>13.54</td>
<td>70.02</td>
<td></td>
<td>67.73</td>
</tr>
<tr>
<td>HOMA</td>
<td>14.75</td>
<td>0.36</td>
<td>13.83</td>
<td>71.40</td>
<td>0.36</td>
<td>68.91</td>
</tr>
<tr>
<td>1/HOMA</td>
<td>13.68</td>
<td>0.68</td>
<td>13.43</td>
<td>69.59</td>
<td>0.68</td>
<td>67.23</td>
</tr>
<tr>
<td>Log(HOMA)</td>
<td>14.07</td>
<td>0.32</td>
<td>13.60</td>
<td>70.26</td>
<td>0.32</td>
<td>68.00</td>
</tr>
<tr>
<td>1/fasting insulin</td>
<td>14.31</td>
<td>0.28</td>
<td>13.92</td>
<td>69.32</td>
<td>0.28</td>
<td>68.32</td>
</tr>
</tbody>
</table>

These error estimates are derived from comparing observed versus predicted GIR or SI_{clamp} from glucose clamp studies as described in MATERIALS AND METHODS (n = 87). P values correspond to statistical comparisons between CVPE or RMSE for QUICKI adjusted for weight versus alternative surrogate indexes adjusted for weight. QCVPE, leave-one-out cross-validation-type root mean squared error of prediction; RMSE, square root of the mean squared error of prediction.
REFERENCES

7-07-RA-80 (to J. K. Kim).
U24-DK-59635 and American Diabetes Association Grants 1-04-RA-47 and by the National Institute of Diabetes and Digestive and Kidney Diseases Grant.

Yale Mouse Metabolic Phenotyping Center and the Penn State Mouse Metabolic Phenotyping Center.

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REFERENCES


