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Christoffersen B, Ribel U, Raun K, Golozoubova V, Pacini G.
Evaluation of different methods for assessment of insulin sensitivity in Göttingen minipigs: introduction of a new, simpler method. Am J Physiol Regul Integr Comp Physiol 297: R1195–R1201, 2009. First published August 26, 2009; doi:10.1152/ajpregu.90851.2008. —The use of animal models in diabetes research requires reliable tests for evaluation of insulin sensitivity and β-cell function. Minipigs are being increasingly used in metabolic research, and the aim of this study was to compare different tests and indexes for evaluation of insulin sensitivity and β-cell function in Göttingen minipigs. Hyperinsulinemic, isoglycemic clamp, intravenous (IVGTT) and oral glucose tolerance tests (OGTT), and a modified insulin tolerance test were performed in minipigs fed either low- or high-energy diet. Furthermore, the reproducibility of IVGTT-derived parameters was assessed. Previously described insulin sensitivity indexes [steady-state glucose infusion rate/glucose concentration/insulin concentration from clamp (M/G/I); oral glucose insulin sensitivity (OGIS) and ISIcomp from OGTT; SI from minimal model analysis of IVGTT; and quantitative insulin sensitivity check index from fasting values] were calculated together with an insulin sensitivity index from the modified insulin tolerance test (ISIITT) and a new simple index (S2) derived from the first 30 min of the IVGTT. β-Cell function was assessed from the IVGTT and the OGTT. Reproducibility of the IVGTT-derived parameters was calculated as median intraindividual coefficient of variation (CV%). M/G/I correlated significantly only with S2 (P < 0.05, r = 0.54), S2 furthermore correlated with SI (P < 0.001, r = 0.81), ISIITT (P < 0.001, r = 0.57), and the two indexes from OGTT, ISIcomp (P < 0.001, r = 0.78) and OGIS (p < 0.05, r = 0.48). No correlation was found between β-cell function indexes from OGTT and IVGTT. The median CV% of the new S2 index was 13. In conclusion, the new simple index of insulin sensitivity, S2, was revealed to be useful for evaluation of insulin sensitivity in pigs.

Glucose tolerance; β-cell function; intravenous glucose tolerance test; oral glucose tolerance test; clamp

TYPE 2 DIABETES IS CHARACTERIZED by glucose intolerance caused by a combination of markedly reduced insulin sensitivity and insulin deficiency relative to the degree of insulin resistance (7, 20). It is therefore of great importance to reliably quantify these two parameters not only in humans but also in animal models of type 2 diabetes. Several methods to evaluate insulin sensitivity and β-cell function have been employed and validated in humans (33) and in rodents (34). These include fasting and dynamic measures obtained from oral (OGTT) and intravenous glucose tolerance tests (IVGTT) as well as insulin tolerance tests (ITT) (4, 5, 27–29, 33, 37). The hyperinsulinemic, isoglycemic or euglycemic clamp is considered the “gold standard” for evaluation of insulin sensitivity (11, 33); however, this test is quite laborious.

Pigs are being increasingly used as an animal model within obesity and diabetes research, and the Göttingen minipig is one of the more extensively utilized strains (19, 22, 23, 26, 36). Various tests and indexes for measuring insulin sensitivity in pigs have been exploited (12, 19, 24, 31, 38, 42), but to our knowledge these have never been validated against the hyperinsulinemic, isoglycemic clamp. This is necessary because this type of animal model is likely to be more employed in studies requiring metabolic measurements.

The aim of the present study was to compare different indexes from OGTT and IVGTT as well as from an ITT with those obtained from the hyperinsulinemic, isoglycemic glucose clamp in Göttingen minipigs of different degrees of obesity and insulin resistance. After this analysis, a new and simple method to evaluate insulin sensitivity was derived from a short IVGTT. The new index emanating from this simple test was validated and its reproducibility assessed.

MATERIALS AND METHODS

Animals. Fourteen female and 11 male Göttingen minipigs 7 wk of age at the beginning of the study were purchased from a microbiologically defined barrier unit [Ellegaard Göttingen Minipigs, Dalmoose, Denmark (www.minipigs.dk)] and used for evaluating four different in vivo tests for the measurement of insulin sensitivity. Other ~6- to 12-mo-old male Göttingen minipigs were used for the reproducibility study. Characteristics of this pig strain have been described in detail previously (10, 15). The pigs were allowed an acclimatization period of 3 wk during which they were gradually introduced to their diets and trained in the experimental procedures. All animals were housed in single pens under controlled conditions, and specially trained personnel cared for the animals. The study was approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Diet and feeding regimen. The 25 pigs in the insulin sensitivity study were fed twice daily with a restricted amount of either low-energy diet (LED; low-fat, high-fiber diet, 6 males and 7 females) or high-energy diet (HED; high-fat, high-sucrose diet, 5 males and 7 females); all pigs had free access to water. The experimental diets, purchased from Brogaarden (Gentofte, Denmark), were given in the milled form (Table 1). The six pigs for the reproducibility study were fed twice daily with a mixture of 140 g of minipig diet from Special Diets Services (SDS; Essex, UK) and 265 g of commercial swine fodder (Antonio, Slangagerup, Denmark) throughout the study.

Implantation of central venous catheters. So that blood samples could be taken without stressing the animals, two central venous catheters (catalog no. C-TPNS-6.5–90-REDO; William Cook, Bjaerverskov, Denmark) were surgically implanted under general anesthesia. The animals were anesthetized intramuscularly with a mixture containing zolazepam and tiletamin (0.81 mg/kg of both zolazepam and tiletamin; Zoletil 50 Vet; ChemVet, Silkeborg, Denmark), ketamine (0.81 mg/kg; Ketaminol Vet, 100 mg/ml; Intervet, Skovlunde, Denmark), xylazine (0.84 mg/kg; Rompun Vet, 20 mg/ml;
Bayer, Lyngby, Denmark), buthophanol (0.16 mg/kg; Torbugesic, 10 mg/ml; Scanvet, Fredensborg, Denmark), and atropine (0.05 mg/kg; Atropin DAK, 1 mg/ml; Nycomed, Roskilde, Denmark). To allow for tracheal intubation, the animals were given 1 mg/kg propofol (Rapinovet, 10 mg/ml; Schering-Plough, Ballerup, Denmark), and during the surgical procedure they were maintained on inhalation of a combination of 1.5–2.5% isoflurane (Rimadyl Vet, 50 mg/ml; Pfizer, Copenhagen, Denmark) and 25 mg/kg dihydrostreptomycin + 20,000 IE/kg benzylpenicillinprocain (Streptocilin Vet, 250 mg dihydrostreptomycin + 200,000 IE benzylpenicillinprocain per ml; Boehringer Ingelheim, Copenhagen, Denmark). Postoperatively, before the end of anesthesia, the animals were given an intramuscular injection of 0.4 mg/kg carprofen (Rimadyl Vet, 50 mg/ml; Pfizer, Copenhagen, Denmark) and 25 mg/kg dihydrostreptomycin + 20,000 IE/kg benzylpenicillinprocain to prevent postsurgical pain and infection.

In vivo tests for evaluation of insulin sensitivity and β-cell function. The tests for evaluation of insulin sensitivity and β-cell function were performed over a period of 3 wk, starting after 3.5 mo of diet feeding and in all cases after an 18-h overnight fast. The pigs used in the reproducibility study had the IVGTT performed twice, 5 days apart.

Mixed-meal oral glucose tolerance test. The OGTT was performed in 23 animals. Immediately after three basal samples (t = −15, −5, and 0 min), pigs were given a mixed-meal glucose load consisting of 2 g/kg glucose (glucose, 500 g/l; SAD, Copenhagen, Denmark) mixed with 12 g of SDS minipig diet; the meal was consumed in ~1 min. Blood samples were then collected at 15, 30, 45, 60, 90, 120, 150, and 180 min.

Intravenous glucose tolerance test. An intravenous glucose bolus of 0.3 g/kg (glucose, 500 g/l; SAD) was administered in 25 pigs over 30 s after collection of basal samples at −20, −10, −5, −2, and 0 min. Blood samples were then collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 30, 45, 60, 90, and 120 min. In the reproducibility experiment blood samples were taken at −10, −5, −2, 0, 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, and 120 min.

Modified insulin tolerance test. The modified insulin tolerance test (mITT), modified after Otis et al. (31), was performed in 22 pigs. After basal samples were taken, an intravenous bolus of 5 μg/kg somatostatin (Sigma S9129; 25 μg/ml; 0.9% sterile saline) was given at −5 min. At the same time, a continuous infusion of 100 mg·kg⁻¹·h⁻¹ somatostatin (Sigma S9129; 25 μg/ml; 0.9% sterile saline) was started and continued for 35 min. An intravenous bolus of 0.5 g/kg glucose (glucose, 500 g/l; SAD) was given at 0 min, and an intravenous bolus of 0.05 U/kg human insulin (100 units standard; Novo Nordisk; diluted to a concentration of 0.5 U/ml in a 50 mM Na-phosphate buffer containing 0.07% polysorbate 20) was given at 15 min. Blood samples were collected at −15, −5, 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 min.

**Table 1. Proximate analysis of the two experimental minipig diets**

<table>
<thead>
<tr>
<th></th>
<th>LED</th>
<th>HED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>10.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>4.3</td>
<td>30.6</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>16.1</td>
<td>16.1</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>10.2</td>
<td>5.6</td>
</tr>
<tr>
<td>Ash, %</td>
<td>4.8</td>
<td>4.7</td>
</tr>
<tr>
<td>Disaccharide, %</td>
<td>5.8</td>
<td>30.6</td>
</tr>
<tr>
<td>Polysaccharide, %</td>
<td>40.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Metabolizable energy, kcal/kg</td>
<td>2,884</td>
<td>4,527</td>
</tr>
</tbody>
</table>

LED, low-energy diet; HED, high-energy diet.

Hyperinsulinemic, isoglycemic clamp. Animals (n = 17) were clamped on their individual fasting blood glucose level (minimum 3.5 mM), which was an average of three fasting blood samples taken immediately before the clamp (−30, −15, and −5 min). Insulin infusion (2 mU·kg⁻¹·min⁻¹, human insulin solution prepared as described above) was initiated at 0 min, and the glucose infusion (12–20 ml/h of a 200 mg/ml solution) was given 5 min afterward. The glucose infusion rate was regulated on the basis of frequent plasma glucose measurements to keep the pigs within ±0.5 mM of their fasting glucose level. Blood samples of 0.8 ml each were collected in heparin-coated glasses for measurement of glucose every 15–20 min: blood was centrifuged for 1 min, and plasma glucose level was immediately measured on a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH) using the glucose oxidase method. Samples for insulin measurement were taken at 15, 30, 60, 90, 120, 150, 180, and 210 min. Glucose infusion rate (M, mg·kg⁻¹·min⁻¹) together with mean glucose (Gm) and mean insulin (Im) concentrations during the last 30 min of the clamp, were used to assess clamp insulin sensitivity (M/Im).

Handling and analysis of blood samples. For each experiment, immediately after collection, blood was transferred to EDTA-coated tubes with 250 KIU/ml Trasylol, kept on ice until centrifugation (10 min at 4°C and 3,500 rpm) 1–1.5 h after sampling. Plasma for glucose analysis was analyzed on the same day using the glucose oxidase method, with 10 μl of plasma in 500 μl of buffer (EBIO plus autoanalyzer and solution; Eppendorf, Germany). Plasma for insulin analysis was pipetted on dry ice and stored at −20°C until analysis.

Porcine insulin (OGTT and IVGTT) was analyzed using an in-house two-site immunometric assay with monoclonal antibodies as capturing and detecting antibodies and using purified porcine insulin for calibration of the assay. The minimal detectable concentration was 3.6 pM, and the upper limit (with no sample dilution) was 1,785 pM. Human insulin (mITT and hyperinsulinemic, isoglycemic clamp) concentration was analyzed using an in-house LOCI sandwich immunnoassay by using two different monoclonal antibodies directed against rat insulin and human insulin, respectively. Cross-reactivity to pig insulin was 15%. The lower limit of quantification was 8 pM, and the upper limit (with no sample dilution) was 3,000 pM. C-peptide concentration was measured using a commercial RIA kit from Linco Research (porcine C-peptide RIA kit catalog no. PCP-22K; St. Charles, MO).

Calculations and statistical analysis. Insulin sensitivity was evaluated from fasting values of glucose and insulin measured on the day of the IVGTT using the quantitative insulin sensitivity check index QUICKI = 1/(log Gb + log Ib) (21), where Gb and Ib are the basal (fasting) levels of glucose and insulin concentration, respectively. From OGTT data, insulin sensitivity indexes previously developed in humans were calculated: ISIcomp = [10,000/(V Gb Ib Gm Im)] (28) and oral glucose insulin sensitivity (OGIS; http://www.isb.cnr.it/ bioing/ogis/home.html) (27). Oral glucose tolerance was determined as the Δ2-h glucose value, and β-cell function was estimated with the insulinogenic index with insulin (Δinsulin₃₀₉₀₃₀ min/Δglucose₃₀₉₀₃₀ min) and C-peptide (ΔC-peptide₃₀₉₀₃₀ min/Δglucose₃₀₉₀₃₀ min), where Δ indicates the relative differences from fasting levels (41).

From the IVGTT, two insulin sensitivity indexes were calculated: the model minimal-derived insulin sensitivity index (8) and a new, simpler index introduced in this study. This new index is defined as

$$K_G = \frac{30 \times K_G}{\int_0^t f(t) dt}$$

K_G is the classic intravenous glucose tolerance index; i.e., the negative slope of the linear regression of the logarithm of glucose vs. time in the 25-min interval from 5 to 30 min. The term at the denominator is
AUC glucose (0–120) values were calculated using the trapezoid rule. Characteristics and metabolic parameters of minipigs were calculated as described above, and furthermore, the AUC insulin (0–120) and glucose concentrations, respectively, from 1 to 5 min. β-Cell function was evaluated as both Δ ACP R C/Δ G m (β-cell sensitivity to glucose) and Δ A IR G/Δ G m (sensitivity of the posthepatic insulin release to glucose).

For the hyperinsulinemic, isoglycemic clamp, the individual M values of the clamp (mg·min⁻¹·kg⁻¹) were divided by the individual steady-state human insulin concentration in the interval from 180 to 210 min and by the individual clamped glucose concentration in the same interval. Thus the final units are also in this case milliliters per minute per micromole per liter per kilogram (ml·min⁻¹·pM⁻¹·kg⁻¹). The new simplified index times Vd is termed S2, and from now on we refer to S1 as the minimal model index times Vd. Vd from the minimal model derives from one of the estimated parameters. When the IVGTT is analyzed only until 30 min, Vd is calculated as the injected glucose dose divided by the mean human insulin concentration in the same interval. This index was also multiplied by Vd (obtained from the clamp, used as the reference gold standard value, it is necessary that the units are the same. The indexes were therefore multiplied by the individual clamped glucose concentration in the interval. This index was also multiplied by Vd (obtained from the minimal model) to obtain the final insulin sensitivity index (ISIITT) in the same units as the other indexes.

Reproducibility study. QUICKI, S2, and Δ A IR G values were calculated as described above, and furthermore, the AUC insulin (0–120) and AUC glucose (0–120) values were calculated using the trapezoid rule. Coefficients of variation (CV%) were calculated as SD/mean×100 of the two measurements for each pig. The median CV% for each parameter was used as a measure of reproducibility.

Statistical analysis. Statistical analysis of the data was done using SAS statistical software (SAS version 9.1 for Windows) with linear regression (Proc Reg in SAS). Statistical outliers and influential observations, identified as observations with a standard residual >3.0 or a Cook’s D value >1, respectively, were excluded from the analysis. All indexes were compared between pigs on LED and HED with Student’s r-test. P values ≤0.05 were considered statistically significant. Data and results are means ± SE.

RESULTS

Characteristics of the minipigs and values of the glucose tolerance, β-cell function, and insulin sensitivity indexes are shown in Table 2, and regression coefficients are shown in Table 3. OGTT and IVGTT time vs. concentration curves are shown in Fig. 1 and 2, respectively, and the most relevant linear regressions are shown in Fig. 3.

The feeding of diets with different energy content to both male and female animals led to the desired quite wide range in body weight and, consequently, in insulin resistance or sensitivity. The mean daily food intake in grams was not different in the two groups [274 ± 8 g in HED and 259 ± 6 in LED, P = not significant (NS)], but as expected, the daily energy intake was significantly higher in the pigs on LED compared with the pigs on LED (1,241 ± 35 vs. 748 ± 15 kcal, P < 0.001).

Glucose tolerance calculated from the OGTT (Δ2-h glucose) and IVGTT (KG) correlated significantly (r = 0.55, P < 0.01), whereas no correlations were found between OGTT and IVGTT β-cell function indexes.

The CV% for glucose and insulin concentrations during the steady-state period of the clamp was 8 and 10%, respectively. The new IVGTT-derived insulin sensitivity index, S2, significantly correlated with M/G/I (r = 0.54, P < 0.05), showing a satisfactory agreement between these two measurements. Minimal model S1 on the contrary, did not correlate with M/G/I, although it correlated significantly with S2. S2 furthermore correlated with ISIcomp, OGIS, and ISIITT (Table 3 and Fig. 3). In addition, ISIITT correlated with S1, ISIcomp, and OGIS, and the two OGTT indexes correlated significantly with each other but not with M/G/I (Table 3).

Significantly lower insulin sensitivity was found in the pigs on HED compared with the pigs on LED with M/G/I (1.0 ± 0.1 vs. 1.7 ± 0.2, P < 0.01), S1 (1.6 ± 0.26 vs. 3.0 ± 0.44, P < 0.05), and ISIITT (1.8 ± 0.2 vs. 3.0 ± 0.2, P < 0.001) but not with S1, ISIcomp, OGIS, or QUICKI (Table 2).

In the reproducibility study an IVGTT was performed twice in a separate group of animals, and the results of the two tests were compared. Body weights at the two test points were

Table 2. Characteristics and metabolic parameters of minipigs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>All Animals</th>
<th>LED Animals</th>
<th>HED Animals</th>
<th>HED vs. LED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>11/14</td>
<td>6/7</td>
<td>5/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>15.1 ± 0.5</td>
<td>13.1 ± 0.2</td>
<td>17.4 ± 0.4</td>
<td>≤0.001</td>
<td></td>
</tr>
<tr>
<td>M/G/I, ml·min⁻¹·pM⁻¹·kg⁻¹</td>
<td>1.4 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>≤0.05</td>
<td></td>
</tr>
<tr>
<td>S1, ml·min⁻¹·pM⁻¹·kg⁻¹</td>
<td>2.0 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>S2, ml·min⁻¹·pM⁻¹·kg⁻¹</td>
<td>2.3 ± 0.3</td>
<td>3.0 ± 0.4</td>
<td>1.6 ± 0.3</td>
<td>≤0.05</td>
<td></td>
</tr>
<tr>
<td>ISIcomp, ml·min⁻¹·pM⁻¹·kg⁻¹</td>
<td>30.1 ± 2.7</td>
<td>33.6 ± 2.4</td>
<td>25.6 ± 2.4</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>OGIS, ml·min⁻¹·pM⁻¹·kg⁻¹</td>
<td>354 ± 29</td>
<td>376 ± 38</td>
<td>324 ± 45</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>ISIITT, ml·min⁻¹·pM⁻¹·kg⁻¹</td>
<td>2.4 ± 0.2</td>
<td>3.0 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>≤0.001</td>
<td></td>
</tr>
<tr>
<td>QUICKI</td>
<td>4.9 ± 0.01</td>
<td>0.50 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Ks, min⁻¹</td>
<td>4.2 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Δ ACP R C/Δ G m, pmol/mmol</td>
<td>47.9 ± 3.8</td>
<td>46.7 ± 4.4</td>
<td>49.2 ± 6.6</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Δ A IR G/Δ G m, pmol/mmol</td>
<td>24.1 ± 2.6</td>
<td>23.2 ± 2.8</td>
<td>25.1 ± 4.5</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Δ2-h Glucose, mg/dl</td>
<td>1.7 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>2.0 ± 0.4</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Δ Glucosemin</td>
<td>89.1 ± 13.1</td>
<td>83.6 ± 16.2</td>
<td>96.3 ± 22.2</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Δ Δ-peptide C peptide Glucosemin</td>
<td>186.0 ± 22.4</td>
<td>179.5 ± 28.1</td>
<td>194.3 ± 38.0</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test. The experimental procedure and formulas for calculating the metabolic parameters, along with their meanings, are reported in MATERIALS AND METHODS. P < 0.05, significant. ns, Not significant.
The CV% was 8.9 for basal insulin, 7.8 for basal glucose, 13.2 for S2, 13.9 for AUC_{insulin(0–120)}, and 6.9 for AUC_{glucose(0–120)}.

DISCUSSION

Minipigs are being used increasingly in metabolic research (6, 12, 19, 25, 26, 31), underlining the necessity of valid methods to evaluate insulin sensitivity in this species. The hyperinsulinemic, isoglycemic or euglycemic clamp is considered the gold standard for evaluation of insulin sensitivity in humans and presumably also in animal models (11, 30). However, because of the laboriousness of this test, several attempts have been made to introduce and validate new and simpler methods to assess insulin sensitivity in humans from both fasting measurements and dynamic tests. Some of the most extensively used alternative indexes are the IVGTT-derived SI from the minimal model (8, 9, 37) and the OGTT-derived ISI_{comp} (28) and OGIS (27), but indexes from ITT also have been exploited (4). All of these indexes have been shown to correlate significantly with clamp measures in humans.

In addition to the evaluation of already existing methods, in this study a new and simple IVGTT-derived insulin sensitivity index, S2, was introduced and validated vs. gold standard glucose clamp and other previously described indexes from OGTT, IVGTT, and mITT in Göttingen minipigs. The proposed S2 index in minipigs is based on the same assumptions of an IVGTT-derived insulin sensitivity index described in humans (14) and in mice (32). The time period of 5–30 min used to calculate KG was chosen because this period avoids the initial mixing phase of glucose and corresponds to the period with a log-linear fall in plasma glucose in these pigs. To calculate S2, KG was divided by the AUC_{insulin(0–30)} to take into account the possible delay in insulin action. The distribution volume, Vd, was included to obtain a measure totally comparable to clamp-derived insulin sensitivity in terms of measurement units

### Table 3. Regression coefficients from linear regression of insulin sensitivity indexes in Göttingen minipigs

<table>
<thead>
<tr>
<th>Index</th>
<th>M/G/I</th>
<th>S1</th>
<th>S2</th>
<th>ISI_{comp}</th>
<th>OGIS</th>
<th>ISI_{ITT}</th>
<th>QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/G/I</td>
<td>ns</td>
<td>0.54*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>S1</td>
<td>0.81‡</td>
<td>0.62†</td>
<td>ns</td>
<td>0.68‡</td>
<td>0.57*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>S2</td>
<td>0.78‡</td>
<td>0.48*</td>
<td>0.42*</td>
<td>0.68‡</td>
<td>0.42*</td>
<td>0.43</td>
<td>ns</td>
</tr>
<tr>
<td>ISI_{comp}</td>
<td>0.42*</td>
<td>0.57*</td>
<td>0.42*</td>
<td>0.68‡</td>
<td>0.42*</td>
<td>0.43</td>
<td>ns</td>
</tr>
<tr>
<td>OGIS</td>
<td>0.43*</td>
<td>0.42*</td>
<td>0.43*</td>
<td>0.68‡</td>
<td>0.42*</td>
<td>0.43</td>
<td>ns</td>
</tr>
<tr>
<td>ISI_{ITT}</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>QUICKI</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are regression coefficients ($r$) from linear regression of indexes obtained using hyperinsulinemic, isoglycemic clamp (M/G/I), OGTT, IVGTT, and modified insulin tolerance test (mITT); $n = 16–25$. See MATERIALS AND METHODS for meanings of all indexes. *$P \leq 0.5$; †$P \leq 0.01$; ‡$P \leq 0.001$. ns, Not significant.

24.2 ± 2.8 and 23.0 ± 2.2 kg ($P < 0.05$). The CV% was 8.9 for basal insulin, 7.8 for basal glucose, 13.2 for S2, 13.9 for AUC_{insulin(0–120)}, and 6.9 for AUC_{glucose(0–120)}.

**Fig. 1.** Glucose (A) and insulin concentrations (B) during a 3-h oral glucose tolerance test (OGTT) in Göttingen minipigs on low-energy (●) and high-energy diet (○). Data are means ± SE ($n = 10–13$).

**Fig. 2.** Glucose (A) and insulin concentrations (B) during a 2-h intravenous glucose tolerance test (IVGTT) in Göttingen minipigs on low-energy (●) and high-energy diet (○). Data are means ± SE ($n = 12–13$).
S2 significantly correlated with clamp-derived M/G/I, indicating its reliability for the evaluation of insulin sensitivity in pigs. Further support of the validity of this index was obtained since, as expected, lower insulin sensitivity was found in the pigs on HED compared with the pigs on LED, with both the S2 index and the M/G/I index (Table 2). In addition, the reproducibility of S2 was satisfactory, being in the same range as that of IVGTT-derived indexes of insulin sensitivity in humans (13, 17, 39).

When compared with other widely used indexes from OGTT, IVGTT, and mITT, S2 also correlated significantly with S1, ISIcomp, OGIS, and ISIITT, but not with QUICKI. Surprisingly, none of the other indexes correlated significantly with clamp insulin sensitivity. This may be due to the inherent differences between the various tests or to the existing differences between the tests in pigs and humans. The glucose clamp quantifies the insulin effect under experimental steady-state conditions and thus does not take into account the delay in insulin action. Depending on the insulin concentration, hepatic glucose production is inhibited to a varying degree; when insulin reaches high concentration levels, primarily peripheral insulin sensitivity is estimated (30). In the present study, the insulin infusion of 2 mU·kg\(^{-1}·\text{min}^{-1}\) given during the clamp was chosen to attain physiological insulin concentrations in the same range as the peak insulin values.

**Fig. 3.** Relationships between different insulin sensitivity indexes in Gottingen minipigs on low-energy (•) and high-energy diet (○). A: insulin sensitivity index from hyperinsulinemic, isoglycemic clamp (M/G/I) vs. new insulin sensitivity index derived from a 30-min IVGTT (S2). B: M/G/I vs. minimal model insulin sensitivity index derived from a 2-h IVGTT (S1). C: M/G/I vs. insulin sensitivity index from the insulin tolerance test (ISIITT). D: S2 vs. ISIITT. E: M/G/I vs. insulin sensitivity index from oral glucose (ISIcomp). F: S2 vs. ISIcomp. G: M/G/I vs. oral glucose insulin sensitivity (OGIS). H: S2 vs. OGIS. P < 0.05, significant. ns, Not significant.
in the OGTT and IVGTT (414 ± 32 vs. 410 ± 39 and 461 ± 91 pM, respectively), making the three tests comparable in that respect. Furthermore, this dose is expected to lead to almost complete inhibition of hepatic glucose output in pigs (18), meaning that M/G/I represents mainly peripheral insulin sensitivity. QUICKI, on the other hand, has been shown to describe almost exclusively hepatic insulin sensitivity. Whereas all of SI, S2, and ISIIT together with the OGTT-derived indexes presumably have both peripheral and hepatic components (1). These differences can affect the degree of correlation, since individuals may have separate hepatic or peripheral insulin resistance, although they are usually well correlated (28, 30).

In addition, the dynamic tests (OGTT, IVGTT, and mITT) are characterized by usually elevated levels of hyperglycemia, and therefore glucose disappearance includes a component due to glucose-mediated glucose disposal, which can be quantified only with a minimal model (2). The weight of this process also may affect the degree of correlation between the dynamic indexes on one hand and M/G/I and QUICKI on the other hand. Furthermore, it probably explains why S2, S1, and ISIIT tend to overestimate insulin sensitivity from the clamp (Fig. 3, A–C), despite having the same units.

Moreover, the OGTT used in minipigs is not completely analogous to that used in humans, since the glucose is given as a mixed meal with a diet rich in fiber. This leads to a slower and more variable gastric emptying and absorption of glucose from the gastrointestinal channel (Fig. 1), and thereby to a smaller and more variable peak in plasma glucose. Thus the OGTT in pigs comprises a weaker metabolic challenge compared with an IVGTT, which may make the OGTT less able to detect small differences in insulin sensitivity. Together with a possible incretin effect on insulin action (3, 40), this may explain the lack of significant difference in insulin sensitivity between the two diet groups and the lack of correlation to the clamp-derived insulin sensitivity. Nonetheless, although probably only able to detect more prominent group differences in insulin sensitivity, the OGTT still may be useful in pigs for providing some information on glucose tolerance, insulin resistance, and insulin secretion given the physiological conditions under which it is performed. The submaximal stimulus on the β-cells during OGTT compared with IVGTT could, in combination with the incretin effect on insulin secretion, also explain the lack of correlation between β-cell function indexes obtained using these two tests in pigs. However, since they were not the major focus of the present study, β-cell function indexes were not compared with the gold standard hyperglycemic clamp.

From a methodological point of view, the insulin and glucose patterns during the IVGTT exhibited less dynamic profiles in pigs compared with humans and dogs (Fig. 2), where the minimal model has been more extensively exploited. Because the estimation of S1 strongly depends on the shape of the concentration patterns (16), it can be speculated that these qualitative differences may lead to an imprecise minimal model estimate of insulin sensitivity in pigs and thus may explain the lack of agreement between S1 and clamp.

**Perspectives and Significance**

Currently, many different nonvalidated tests and indexes are used to describe insulin sensitivity in (mini)pigs in situations where hyperinsulinemic clamp is not performed (which is the majority of the studies at present). This may make different studies, feeding regimens, and effects of drug candidates difficult to compare. By introducing a quick, simple, and validated intravenous method, we hope to make it “first choice” in settings where clamp is not performed. Thereby, the published studies in (mini)pigs would become more uniform and easier to compare, which would be a huge advantage. The new insulin sensitivity index, S2, is simple to obtain from a short IVGTT, and furthermore, it is the only index that appears to correlate with clamp in minipigs. The new index is based on general physiological principles not depending on the exact shape of the glucose and insulin curves and is therefore likely to be applicable not only in Göttingen minipigs but also in other pigs and species. Indeed, this simplified test already has been shown to be applicable in humans (14) and mice (32). The applicability of S2 in diabetic animals with low or no endogenous insulin secretion remains to be clarified, but the method may be further developed with exogenous insulin infusion as has been done with the insulin-modified IVGTT in humans (35).

In conclusion, the new S2 insulin sensitivity index, based on a short IVGTT, significantly and exclusively correlates to the insulin sensitivity index from the hyperinsulinemic, isoglycemic clamp. The S2 index is simple and useful for evaluation of insulin sensitivity in pigs.

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**REFERENCES**


