Reappraisal of the intravenous glucose tolerance index for a simple assessment of insulin sensitivity in mice

Giovanni Pacini,1 Maria Ahre´n,2 and Bo Ahre´n2

1Metabolic Unit, Institute of Biomedical Engineering, National Research Council, Padova, Italy; and 2Department of Clinical
Sciences, Division of Medicine, Lund University, Lund, Sweden

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Am J Physiol Regul Integr Comp Physiol 296: R1316–R1324, 2009. First published February 11, 2009; doi:10.1152/ajpregu.90575.2008.—Mice are increasingly used in studies where measuring insulin sensitivity (IS) is a common procedure. The glucose clamp is labor intensive, cannot be used in large numbers of animals, cannot be repeated in the same mouse, and has been questioned as a valid tool for IS in mice; thus, the minimal model with 50-min intravenous glucose tolerance test (IVGTT) data was adapted for studies in mice. However, specific software and particular ability was needed. The aim of this study was to establish a simple procedure for evaluating IS during IVGTT in mice (CSI). IVGTTs (n = 520) were performed in NMRI and C57BL/6J mice (20–25g). After glucose injection (1 g/kg), seven samples were collected for 50 min for glucose and insulin measurements, analyzed with a minimal model that provided the validated reference IS (SI). By using the regression CSI = α1 + α2 × KG/AUC0, where KG is intravenous glucose tolerance index and AUCo is the dynamic area under the curve, IS was calculated in 134 control animals randomly selected (regression CSI vs. SI: r = 0.66, P < 0.0001) and yielded α1 = 1.93 and α2 = 0.24. KG is the slope of log(glucoses20) and AUC0 is the mean dynamic area under insulin curve in the IVGTT. By keeping fixed α1 and α2, CSI was validated in 143 control mice (4.7 ± 0.2 min·μU·ml−1, virtually identical to SI: 4.7 ± 0.3, r = 0.89, P < 0.0001); and in 123 mice in different conditions: transgenic, addition of neuropeptides, incretins, and insulin (CSI: 6.0 ± 0.4 vs. SI: 6.1 ± 0.4, r = 0.94, P < 0.0001). In the other 120 animals, CSI revealed its ability to segregate different categories, as does SI. This easily usable formula for calculating CSI overcomes many experimental obstacles and may be a simple alternative to more complex procedures when large numbers of mice or repeated experiments in the same animals are required.

insulin resistance; glucose effectiveness; animal model; pituitary adenylate cyclase-activating polypeptide; glucagon-like peptide-1; intravenous glucose tolerance test

THE EUGLYCEMIC HYPERINSULINEMIC glucose clamp (22) is considered the reference method for measuring insulin sensitivity in humans, while it seems to present several drawbacks in interpreting the results on insulin sensitivity in mice (8). In addition, the technique in itself has some limitations: it is labor intensive, requires a complicated experimental setting, cannot be carried out in a large number of animals, and, most importantly, cannot be repeated in the same mouse for technical reasons. Furthermore, the euglycemic hyperinsulinemic glucose clamp does not provide any information on insulin secretion. This is a weakness, because insulin sensitivity and secretion need to be calculated together for an accurate estimation of insulin effectiveness (6, 19). These obstacles have been overcome by adapting to mice data the minimal model of glucose disappearance. This method has been validated against the clamp (23), which, despite the above limitations, is still accepted as the gold standard in the measurement of insulin resistance also in rodents. The minimal model has been shown to be a valid tool to estimate insulin sensitivity in mice after an intravenous glucose tolerance test (IVGTT) lasting 1 h with seven samples and has been successfully applied to mice studies (among many others, see for instance, Refs. 2, 3, 5, 7, 11, 26).

The IVGTT has the advantage over the euglycemic clamp of being a dynamic test. Rodents, in fact, do not have a true physiological fasting state, and maintaining a nonphysiological steady-state condition may lead to incorrect interpretations of the results (8). Further advantages over the clamp are that the IVGTT can be used in many animals and that it can be repeated in the same animal several times. Of special interest is the fact that in addition to measuring insulin sensitivity and secretion, the IVGTT with modeling analysis also quantifies the insulin-independent glucose disappearance (glucose effectiveness) (10). However, the approach with the minimal model, though simpler than the clamp, needs the use of specific software for which a particular ability and skill of the operator is necessary. There is thus a need for making simpler the procedures for assessment of metabolic parameters (20).

In humans, Galvin et al. (13) proposed an easily computable explicit formula of glucose kinetics for obtaining insulin sensitivity from glucose and insulin IVGTT data. The aim of our study was, therefore, to verify whether similar formulas are able to provide insulin sensitivity and glucose effectiveness from glucose and insulin concentration data during a standardized IVGTT in mice. To that end, glucose kinetics in these animals, after intravenous glucose administration, was first evaluated in detail. Then, the “new” formula was validated by comparing the results with those obtained with the standard minimal model, assumed as reference method, being validated earlier. Glucose and insulin responses were analyzed under a variety of experimental conditions, which included coadministration with glucose of the glucocorticoid hormone glucagon-like peptide-1 (GLP-1), the GLP-1 receptor antagonist exendin9-39, the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) of which two forms were used (PACAP27 and PACAP38), and the adipocyte hormone acylation-stimulating protein (2, 5, 11). Furthermore, glucose and insulin responses to intravenous glucose were also analyzed in mice fed a diet supplemented with 80% conjugated linoleic acid and in mice genetically deleted of the neuropeptide galanin (3, 26).
METHODS

General Design

A total of 542 experiments were performed in nonfasted NMRI and C57BL/6J mice (Taconic, Skensved, Denmark) weighing 20–25 g and kept on a 12:12-h light-dark schedule (lights on at 0600). In one experimental series, IVGTT was followed by frequent sampling of 17 samples, whereas in another experimental series, the regular seven-sample IVGTT with modeling of data was undertaken (23). All experiments were performed in late morning without food being removed from the cages. The experimental procedures were approved by the Ethics Committee of Lund University. The normal animals, fed with a standard pellet diet (fat 11.4%, carbohydrate 62.8%, protein 25.8%, total energy 12.6 kJ/g) and tap water ad libitum, were anesthetized with an intraperitoneal injection of midazolam (Dormicum, 0.4 mg/mouse; Hoffman-La Roche, Basel, Switzerland) and a combination of fluanisone (0.9 mg/mouse) and fentanyl (Hypnorm; 0.02 mg/mouse; Janssen, Beerse, Belgium). The anesthesia persisted for 1 h. During the experimental procedure, the animals were kept on a heating pad. The experiments were divided into different groups: IVGTT with glucose administration alone or IVGTT with glucose administered together with other compounds.

IVGTT

A blood sample was taken, and thereafter, an intravenous injection was given in a tail vein. The injection consisted of glucose alone or glucose together with other compounds. Injection of glucose was undertaken as d-glucose (10 g/dl; Sigma-Aldrich, St Louis, MO) injected intravenously over 3 s at a dose of 1 g/kg in a tail vein without flushing of the 27-gauge needle after injection. A glucose dose was chosen because, in previous experiments, we observed a rise and peak in insulin only at 1 min, and rarely at 5 min, when giving lower doses of glucose, such as 0.3 or 0.25 g/kg (not shown). Blood samples, according to the different schedules, were taken from the retrobulbar, intraorbital, and capillary plexus and put into a 100-µl pipette that had been prerinse din heparin solution (100 U/ml in 0.9% NaCl; Lõvens, Ballerud, Denmark). The total injected volume load was 10 µl/g body wt.

Evaluation of Glucose Disappearance Rate

For estimation of glucose disappearance rate, 22 IVGTTs were performed in which 17 blood samples (full protocol) were taken for determination of glucose. The IVGTTs were undertaken in mice anesthetized as above. In 16 animals, glucose was given alone, as described above, while in six animals glucose was given together with 1 nmol/kg of GLP-1 (Peninsula Laboratories Europe, Merseyside, UK). This incretin is a potent insulinotropic agent in mice and markedly increases insulin concentrations during the experiment (5). Samples were collected at times $t = -1, 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10, 12.5, 15, 20, 25, 30, 40, 50$ min. The last sample was at 50 min to avoid possible influence on the measurements of awakening from anesthesia. Glucose disappearance was described by logistically transforming the glucose concentration. This allows the identification of three different phases (see RESULTS) typifying the glucose pattern. The first two phases are fitted with a double exponential curve $A \cdot \exp(-(a \cdot t)) + B \cdot \exp(-(b \cdot t))$ to characterize the single patterns, where $A$ and $B$ are extrapolated zero time values assumed by the two exponential curves. The net glucose elimination rate was evaluated from parameter $b$: i.e., the slope of the logarithm of glucose concentration vs. time $(i)$ in the interval detected as the main elimination phase. This parameter, also called intravenous glucose tolerance index $(K_D)$, when calculated as $-100 \times b$, has been frequently used in humans and mice (e.g., Ref. 16 and 23, respectively) to characterize the glucose pattern following a bolus glucose injection.

Insulin Sensitivity with Minimal Modeling Analysis

A total of 473 IVGTT with minimal model analysis was performed. Data of all these experiments were derived from previous investigations (2, 3, 5, 11, 26) and were reanalyzed for the purposes of the present study. In particular, 277 animals received only glucose (control), while the other animals also received other substances together with glucose (see below). Blood was sampled at $t = -1, 1, 5, 10, 20, 30, 50$ min after injection (75 µl each). The first sample was at 1 min, because by that time the insulin response to the glucose bolus is already clearly expressed. Insulin and glucose IVGTT data were analyzed with the minimal model technique (9). This procedure has been detailed in many previous publications (e.g., Ref. 21). Briefly, the model assumes first-order glucose kinetics with nonlinear control by insulin and accounts for the effect of insulin and glucose itself on glucose disappearance following exogenous glucose injection. The model provides two main parameters: the insulin sensitivity index $(S_I)$ (min$^{-1}$µU$^{-1}$ml$^{-1}$), which describes the glucose disappearance from plasma per se without any change in dynamic insulin ($I_d$) i.e., at basal insulin $(I_b)$. With the seven-sample IVGTT, $K_D$ was calculated in the interval 5–20 min after glucose injection (23). Model parameters were log transformed before estimation to guarantee positive values for back-transformed parameters and to reduce the effects of the possibly large between-animal variability.

Assays

After immediate centrifugation, plasma was separated and stored at $-20^\circ$C until analysis. Plasma insulin was determined in single samples radioimmunoochemically with the use of a guinea pig anti-rat insulin antibody, $[^{25}]$H-labeled human insulin as tracer, and rat/mouse or human insulin as standard (Linco Research, St. Charles, MO). There was a complete overlap in the assays, showing that the affinity was the same for rat/mouse and human insulin standard (data not shown); therefore no correction factor was used. Free and bound radioactivity were separated by use of an anti-IgG (goat anti-guinea pig) antibody (L disobio). The sensitivity of the assay is 2 µIU/ml, and the coefficient of variation is < 3% within assays and < 5%, between assays. Plasma glucose was determined with the glucose oxidase method (coefficient of variation < 1%).

Calculations and Statistics

The area under the curve (AUC) of insulin concentration was calculated using the trapezoidal rule. Data and results are reported as means ± SD or means ± SE as designated. Statistical comparisons within or between any group or subgroups were performed with Student’s $t$-test (paired or unpaired according to the specific comparison) after checking for normal distribution. Accuracy of the minimal model estimates was evaluated from the coefficient of variation as fractional SD, calculated from the variance values in the main diagonal of the inverse of the Fisher’s information matrix.

Simple Estimation of Insulin Sensitivity and Glucose Effectiveness

Derivation of the formula for insulin sensitivity. The intravenous glucose tolerance index $(K_D)$ describes glucose disappearance both under the effect of dynamic insulin (related to insulin sensitivity) and on glucose disposal at basal insulin (glucose effectiveness), the latter including also the glucose-dependent processes (1, 27, 10). Previously, we have shown how the model-independent $K_D$ is related to the physiological parameters (insulin sensitivity, $S_I$ and glucose effectiveness, $S_G$) obtained in mice by analyzing IVGTT data with the minimal model (23). In particular, we evaluated that $K_D$ is linearly related to a function of insulin sensitivity and secretion (the “disposition index” $S_I \times $ AUC). In addition, Bergman (9) has clearly demonstrated that the insulin sensitivity index depends upon the glucose disappearance rate and the suprabasal concentration that follows the glucose stimulation.
Here, we applied a similar approach to relate $K_G$ to the metabolic parameters, and this justifies the structure of a formula, similar to that proposed in humans by Galvin et al. (13), which avoids complex computer modeling. Therefore, to segregate the component of $K_G$ due to the dynamic insulin action, we assume that an index of insulin sensitivity should be linearly related to the ratio of $K_G$/AUC$_D$, as already shown (23). AUC$_D$ is defined as the dynamic area under the insulin curve in the IVGTT interval 0–50 min divided by the length of the interval, i.e.

$$\frac{1}{50} \int_0^{50} [I(t) - I_{50}] \, dt$$

This can be easily calculated as the total AUC minus the basal area $= I_{50} \times 50$, and represents also the mean suprabasal insulin concentration, since $I_{50}$ is the insulin measured 50 min after the glucose injection in the IVGTT. It has been chosen because it is the reference “basal” level of insulin ($I_{50}$) commonly used by the minimal model to estimate $S_I$. The units of $K_G$/AUC$_D$ are therefore the same as $S_I$ i.e., min$^{-1}$μU$^{-1}$ml. Defining $CS_I$ as the value of insulin sensitivity related to $K_G$/AUC$_D$, we can write the formula

$$CS_I = \alpha_1 + \alpha_2 \frac{K_G}{AUC_D} \quad (1)$$

The index $S_I$ from the minimal model is used as the reference value, since it has been shown to exhibit a good correlation in mice with the glucose clamp (23) and to be a valid index of insulin sensitivity in several studies (9, 24). When $CS_I$ and $K_G$/AUC$_D$ were substituted with the previously evaluated $S_I$, $K_G$, and AUC$_D$, respectively, of 134 control mice (control group 1) randomly selected among 277 experiments, the Eq. 1 was used to estimate parameters $\alpha$. This procedure was repeated five times, with a random selection of $\sim 50\%$ of the 277 IVGTT and the parameters $\alpha$ resulted not different from those found the first time (not shown). Therefore, we assumed, for the following procedure, the $\alpha$-values obtained in the 134 animals of the first round.

Sample size. An a posteriori study on the minimum number of animals required for reliable estimation of the parameters was carried out by using the Monte-Carlo simulation method. A nonparametric bootstrap analysis (17) was performed. For sample sizes ranging from 10 to 140 (maximum size of group 1), we randomly selected a subsample of the original values and computed the SEs of the two estimates of $\alpha_1$ and $\alpha_2$. This was repeated 1,000 times.

Validation of the formula. Once these $\alpha$-values are validated, Eq. 1 can be used to assess insulin sensitivity from simple calculations on IVGTT data without the use of complex mathematical modeling. Thus, it is necessary to validate the correctness of the estimated parameters. To do this, two approaches were taken: one was the comparison between $CS_I$ (obtained with the estimated $\alpha$-values kept fixed) and the reference minimal model $S_I$ in the remaining 143 control (group 2) animals and in other 123 (group 3) animals that underwent different protocols. The other approach was to verify that $CS_I$ was able to detect the differences in insulin sensitivity already seen in previous studies where $S_I$ from the minimal model was used.

Of the 123 animals of group 3, 26 received PACAP at various doses together with glucose (11), 11 received acylation-stimulating protein at 50 nmol/kg (2), and 15 received exenatide at 30 nmol/kg (5). A diet with 80% conjugated linoleic acid was supplemented for 1 wk to 27 mice (26), and the last two groups consisted of 23 galanin-gene-deleted mice and 21 wild-type mice that were injected only with glucose (3).

Regarding the observation of the difference in insulin sensitivity, we have previously studied the effect of PACAP27, PACAP38, GLP-1, and the galanin gene-deletion on insulin sensitivity, and we refer to previous publications about the nature, physiology, and effects of these substances (3, 5, 11). To check whether the difference in the parameters we observed with the minimal model analysis is confirmed by $CS_I$, four studies were repeated 1) PACAP38 at the dose of 1.3 nmol/kg was given in 24 mice; 2) PACAP27 at the dose of 1.3 nmol/kg in 15 animals, both compared with a same number of control animals; 3) GLP-1 at the dose of 10 nmol/kg in 24 animals compared with 23 control mice and 10 mice injected with insulin at 0.1 U/kg; and 4) comparison between galanin gene-deleted mice and the corresponding wild-type mice. These last experiments, without control or wild-type animals, were also used for a further validation of the method. $S_I$ and $K_G$/AUC$_D$ of the 97 animals allowed the direct estimation of the $\alpha$-parameters to be compared with those obtained in the 134 control (group 1).

Estimation of glucose effectiveness. The glucose disappearance, as estimated from the IVGTT, is a combination of the effort of dynamic insulin (strictly related to insulin sensitivity) and glucose disappearance per se at $I_0$ (glucose effectiveness) (1, 10). Therefore, $K_G$ related to the whole insulin, including the basal one (AUC$_T$), must depend upon both $S_I$ and $S_C$, according to the relationship: $K_G$/AUC$_T$ = $m_1$ + $m_2$ × $S_I$ + $m_3$ × $S_C$/I$_50$, where $m_1$ is the linear coefficient. $S_C$ is divided by $I_0$, to have consistent units, as already done in humans (15). Defining $CS_G$ as the value of glucose effectiveness related to both insulin sensitivity and $K_G$/AUC$_T$, and rearranging the terms, the following equation can be used to estimate the $\lambda$-parameters with multiple regression

$$CS_G = \lambda_1 + \lambda_2 S_I + \lambda_3 K_G/AUC_T \quad (2)$$

when $S_I$ and $K_G$/AUC$_T$ were substituted with the previously evaluated $S_I$, $K_G$, and AUC$_T$, respectively, of the 134 control (group 1). Because $CS_G$ also needs to be validated, the first validating step has been that of comparing $CS_G$ to $S_C$/I$_50$ in these animals, once $S_I$ in Eq. 2 has been substituted with $CS_I$, estimated previously for the same animals. A further validation has then been the comparison between $CS_G$ and $S_C$/I$_50$ keeping the $\lambda$-values fixed in the other 143 animals (group 2), where $S_C$/I$_50$ from the minimal model was known. Parameters $\lambda$ were also estimated from the 97 experiments with PACAP, GLP-1, and galanin for comparison with those obtained in the 134 control (group 1).

RESULTS

Intravenous Glucose Disappearance

Figure 1A shows the average glucose pattern after injection of glucose alone and glucose plus GLP-1. Glucose levels peak after 30 s, followed by a two-phase elimination rate that is enhanced by GLP-1. Fig. 1B describes the glucose levels in both sets of experiments after logarithmic transformation and clearly shows that three phases can be identified. The first phase, which is the mixing or distribution phase of glucose, is fast and lasts for 3 min in both cases (slope for glucose only: $a = -0.141 \pm 0.013$ min vs. $-0.156 \pm 0.008$ of glucose + GLP-1; mean ± SE, $P = 0.522$), whereas the second one, more prolonged, is the main elimination phase and lasts until 25 min. The slope of the line for this phase was increased by GLP-1 ($b = -0.063 \pm 0.003$ vs. $-0.035 \pm 0.02$ of glucose only; $K_G = 6.33 \pm 0.26$ and $3.53 \pm 0.18/%; P < 0.000001$). The half-life of glucose was therefore shorter with GLP-1 ($11 \pm 2$ vs. $18 \pm 4$ min; $P = 0.0003$). The third phase, the stabilization phase, represents glucose concentration reaching and maintaining the equilibration steady state.

Since the glucose disappearance $K_G$ from the common seven-sample IVGTT is necessarily calculated only with the available samples in the identified main elimination phase that are the samples at 5, 10, and 20 min, we compared the $K_G$ obtained with these three samples with the full protocol $K_G$ calculated as $-100 \times b$. The two different measurements highly correlated ($r = 0.9, P < 0.00001$). The three-sample $K_G$
was 3.65 ± 0.22%/min with glucose only (P = 0.449 vs. full protocol K\textsubscript{G}) and 6.77 ± 0.35 with GLP-1 (P = 0.09). The two three-sample K\textsubscript{G} reflected the differences of glucose disappearance with and without GLP-1 (P = 0.000003). The three-sample K\textsubscript{G} was then reliably used for the following analysis.

**Determination of CS\textsubscript{I} from Equation 1**

The minimal model estimated and calculated parameters in all 277 normal mice are shown in Table 1. Equation 1 was applied in 134 randomly selected animals (group 1) and the linear regression between SI and G was then reliably used for the following analysis. The Monte Carlo analysis indicated that for endpoints similar to those used in our study, 80 animals give rise to an equivalent estimate, although a number of experiments as low as 40 would have been sufficient for reliable estimation (P < 0.05) of the parameters.

Linear regression between SI and K\textsubscript{G}/AUC\textsubscript{D} of the 97 experiments with PACAP, GLP-1, and galanin (r = 0.95, P < 0.0001) yielded \(\alpha_1 = 1.104 ± 0.199\) and \(\alpha_2 = 0.267 ± 0.009\) not different (P > 0.1) from those obtained in group 1 and shown in Table 2.

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**Table 1. Parameters from IVGTT in mice**

<table>
<thead>
<tr>
<th>Experiments</th>
<th>No.</th>
<th>Mice</th>
<th>K\textsubscript{G}, %/min</th>
<th>SI, (10^{-4}) min (mU/ml)</th>
<th>Dynamic Insulin Area, (10^{-5}) min (mU/ml)</th>
<th>Total Insulin Area, (10^{-2}) min (mU/ml)</th>
<th>SG/I50, %</th>
<th>CSG, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>All control mice</td>
<td>277</td>
<td>134</td>
<td>2.90 (0.30–8.88)</td>
<td>49 (2–109)</td>
<td>2.08 (0.10–24.60)</td>
<td>2.23 (0.23–19.22)</td>
<td>1.4 (1.0–11.8)</td>
<td>16.2</td>
</tr>
<tr>
<td>Group 1 (control)</td>
<td>277</td>
<td>134</td>
<td>2.90 (0.30–8.88)</td>
<td>49 (2–109)</td>
<td>2.08 (0.10–24.60)</td>
<td>2.23 (0.23–19.22)</td>
<td>1.4 (1.0–11.8)</td>
<td>16.2</td>
</tr>
<tr>
<td>Group 2 (control)</td>
<td>277</td>
<td>134</td>
<td>2.90 (0.30–8.88)</td>
<td>49 (2–109)</td>
<td>2.08 (0.10–24.60)</td>
<td>2.23 (0.23–19.22)</td>
<td>1.4 (1.0–11.8)</td>
<td>16.2</td>
</tr>
<tr>
<td>Group 3 (control)</td>
<td>277</td>
<td>134</td>
<td>2.90 (0.30–8.88)</td>
<td>49 (2–109)</td>
<td>2.08 (0.10–24.60)</td>
<td>2.23 (0.23–19.22)</td>
<td>1.4 (1.0–11.8)</td>
<td>16.2</td>
</tr>
<tr>
<td>Group 1 (various protocols)</td>
<td>123</td>
<td>43</td>
<td>2.90 (0.30–8.88)</td>
<td>49 (2–109)</td>
<td>2.08 (0.10–24.60)</td>
<td>2.23 (0.23–19.22)</td>
<td>1.4 (1.0–11.8)</td>
<td>16.2</td>
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<td>16.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; numbers in parenthesis are the range. Tolerance index (K\textsubscript{G}), reference basal insulin (I\textsubscript{50}), and total and dynamic area under the insulin curve were directly calculated from intravenous glucose tolerance test (IVGTT) data, while insulin sensitivity and normalized glucose effectiveness were estimated (SI and SG/I\textsubscript{50}, respectively) with the minimal model or derived (CSI and CSG) with the simple formulas (Eqs. 1 and 2, respectively, and are to be compared with SI and SG/I\textsubscript{50}, respectively.
Determination of CSG from Equation 2

A multiple regression of the known $S_I$ and $K_G/AUCT$ in the 134 control experiments of group 1 ($r = 0.62, P < 0.0001$) yielded the parameters (Table 2). These were validated by applying Eq. 2 with fixed $\lambda$ in the 143 control of group 2 to calculate CSG from the known $K_G/AUCT$. CSG was 16.1 $\pm$ 0.7 min$^{-1}\cdot\mu$U$^{-1}\cdot$mL, virtually identical to the minimal model $S_C/I_b$ (16.3 $\pm$ 1.2, $P = 0.865$), assumed again as the reference value. Also these two measurements highly correlated ($r = 0.81, P < 0.0001$).

Further Validation of the Simplified Formula

Equations 1 and 2 with fixed $\alpha$- and $\lambda$-values were also applied in different sets of mice (123 animals of group 3), where various substances were added to glucose to create different situations affecting both insulin sensitivity and secretion. Also, in these cases, simple CSI and the real $S_I$ results were similar, as well as CSG and $S_C/I_b$ (Table 1). Fig. 3A shows the comparison between the estimated and the calculated insulin sensitivity ($r = 0.94$), and Fig. 3B shows that between the normalized glucose effectiveness ($r = 0.90$). In both cases, the correlation was highly significant ($P < 0.0001$). Parameters $\lambda$ obtained with multiple regression ($r = 0.57, P < 0.0001$) in the 97 experiments with PACAP, GLP-1, and galanin ($\lambda_1 = 10.762 \pm 2.257, \lambda_2 = -2.472 \pm 0.557, \lambda_3 = 5.611 \pm 0.875$) were not different ($P > 0.1$) from those in Table 2.

Table 2. Coefficients of Eqs. 1 and 2 obtained with regression among known variables

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Units</th>
<th>Estimated Value</th>
<th>SE of the Estimate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_1$</td>
<td>$10^4$ min$^{-1}\cdot\mu$U$^{-1}\cdot$mL</td>
<td>1.929</td>
<td>0.302</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>dimensionless</td>
<td>0.235</td>
<td>0.024</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>$10^4$ min$^{-1}\cdot\mu$U$^{-1}\cdot$mL</td>
<td>8.130</td>
<td>2.179</td>
<td>$&lt;0.0003$</td>
</tr>
<tr>
<td>$\lambda_2$</td>
<td>dimensionless</td>
<td>-1.988</td>
<td>0.434</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>$\lambda_3$</td>
<td>dimensionless</td>
<td>4.211</td>
<td>0.472</td>
<td>$&lt;0.0001$</td>
</tr>
</tbody>
</table>

Evaluation of Simplified Formulas in Different Mouse Strains

When applied separately in the two different strains (i.e., C57BL/6J and NMRI, 89 and 188 control animals, respectively), no difference was found comparing both CSI and CSG with the respective $S_I$ and $S_C/I_b$, and the correlation was statistically significant (Table 3).

Use of the Simplified Formulas in Other Studies

Regarding PACAP and GLP-1 studies, in Table 4 the values of insulin sensitivity and normalized glucose effectiveness obtained with the simplified Eqs. 1 and 2 are shown. The previous results on reduced insulin sensitivity and unchanged glucose effectiveness with both PACAP27 and PACAP38 were confirmed (see Table 3 of Ref. 11). In the GLP-1 study, the simple formulas were able to show the same differences of insulin sensitivity in the various subgroups; however, CSG results were underestimated compared with the minimal model-derived $S_C/I_b$. To verify the ability of detecting possible changes in $S_C/I_b$ with lower insulin, we injected insulin at the dose of 0.1 U/kg. The resulting AUC$_T$ was 3.51 $\pm$ 0.44.
Table 3. Comparison between $S_T$ and $CS_I$ (both $10^4$ min$^{-1}$·μU$^{-1}$·ml) in the two mouse strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. Mice</th>
<th>$S_T$</th>
<th>$CS_I$</th>
<th>$P$ Value</th>
<th>$S_G/I_{50}$</th>
<th>$CS_G$</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>89</td>
<td>5.91±0.52</td>
<td>6.05±2.78</td>
<td>0.702</td>
<td>22.4±19.8</td>
<td>21.3±9.4</td>
<td>0.537</td>
</tr>
<tr>
<td>NMRI</td>
<td>188</td>
<td>3.84±2.487</td>
<td>3.86±1.46</td>
<td>0.910</td>
<td>13.2±4.7</td>
<td>13.5±6.0</td>
<td>0.713</td>
</tr>
</tbody>
</table>

Values are means ± SD.

μU·ml$^{-1}$·min ($P = 0.045$ vs. $2.80 ± 0.13$ of the relative control animals), while it was $17.46 ± 1.49$ with GLP-1. No changes were observed in $CS_I$ of control animals. Results of the galanin study showed that the outcomes with the simplified formulas were similar to those obtained with the minimal model ($P = 0.6$). $CS_I$ confirmed the slight, though not significant, increase of insulin sensitivity in the knockout mice compared with wild-type animals (Table 4).

**DISCUSSION**

This study presents simple explicit formulas for the determination of two important metabolic parameters, such as insulin sensitivity and glucose effectiveness, in mice. Our approach, which can be considered an extension of a previously presented method (13), is based on the general definition of insulin sensitivity; i.e., changes in glucose disappearance over changes in insulin concentration. The glucose disappearance rate following intravenous glucose administration was studied during an IVGTT with frequent sampling to characterize the various phases of the test, which were three. The first phase is the distribution, which lasts for approximately 3 min, and is followed by the main elimination phase. This phase is then followed by the stabilization phase, which represents glucose concentrations reaching and maintaining the equilibration steady state. For estimation of insulin sensitivity, the true elimination phase is used, and the frequent sampling revealed this to last from minute 3 until minute 25. Logarithmic transformation of the glucose data during this time interval was fitted by a line the slope of which is the glucose tolerance index ($K_G$). $K_G$ has been used previously in several studies for indirect estimation of glucose elimination both in humans (16) and in mice (23). The first part of the present study validated this technique, demonstrating the robustness of $K_G$ estimated by the samples between 5 and 20 min. In fact, this was identical to the $K_G$ estimated as the slope of the entire study period with the frequent sampling.

For the calculation of insulin sensitivity, we assumed that $S_T$ from the minimal model is linearly related to the ratio between $K_G$ and the dynamic insulin AUC during the 50-min time period. Other combinations of direct measurements were tested (not shown), but Eq. 1 revealed the best approximation. We then proceeded by estimating the constants $\alpha_1$ and $\alpha_2$, in a large cohort of control animals randomly chosen among a series of experiments previously carried out in different conditions. This allowed an unbiased set of $S_T$, spanning from low ($\sim 0.3 10^{-4}$ min$^{-1}$·μU$^{-1}$·ml) to quite high ($>15 10^{-4}$ min$^{-1}$·μU$^{-1}$·ml) values. The resulting formula, with $\alpha_1$ and $\alpha_2$ kept fixed, was applied to another set of experiments, and the resulting $CS_I$ was virtually identical to the reference $S_T$. After further validation in other animals reflecting different experimental conditions and physiological situations, we feel confident in proposing to calculate the insulin sensitivity index from a seven-sample IVGTT in mice by the formula $CS_I = 1.93 + 0.24 \times (K_G/AUC_D)$. The validity of this formula lies in its significant correlation with $S_T$ as determined by minimal modeling in a large number of experiments performed under a variety of experimental conditions, together with the previously demonstrated high correlation between $S_T$ and insulin sensitivity determined by the euglycemic hyperinsulinemic clamp technique (23). In addition, starting from experiments with PACAP, GLP-1, and galanin, $\alpha$- and $\lambda$-constants were estimated and were not

Table 4. Insulin sensitivity and normalized glucose effectiveness from IVGTT in mice, estimated ($S_T$ and $S_G/I_{50}$, respectively) with the minimal model and calculated ($CS_T$ and $CS_G$) with the simple formulas (Eqs. 1 and 2)

<table>
<thead>
<tr>
<th>Experiments</th>
<th>No. Mice</th>
<th>$S_T$ 10$^4$ min$^{-1}$·μU/ml</th>
<th>$CS_T$ 10$^4$ min$^{-1}$·μU/ml</th>
<th>$S_T$ 10$^4$ min$^{-1}$·μU/ml</th>
<th>$CS_T$ 10$^4$ min$^{-1}$·μU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>PACAP38</td>
<td>24</td>
<td>1.76±0.34</td>
<td>2.59±0.08</td>
<td>10.4±1.3</td>
<td>11.0±0.4</td>
</tr>
<tr>
<td>Control of PACAP38</td>
<td>24</td>
<td>3.97±0.42</td>
<td>3.78±0.22</td>
<td>13.0±3.1</td>
<td>13.8±1.4</td>
</tr>
<tr>
<td>$P$ value&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>0.00017</td>
<td>0.00001</td>
<td>0.054</td>
<td>0.053</td>
</tr>
<tr>
<td>PACAP27</td>
<td>15</td>
<td>1.25±0.20</td>
<td>2.54±0.09</td>
<td>8.1±1.4</td>
<td>9.6±0.6</td>
</tr>
<tr>
<td>Control of PACAP27</td>
<td>16</td>
<td>2.85±0.49</td>
<td>3.05±0.15</td>
<td>6.8±1.3</td>
<td>10.7±0.8</td>
</tr>
<tr>
<td>$P$ value&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>0.00297</td>
<td>0.00889</td>
<td>0.496</td>
<td>0.305</td>
</tr>
<tr>
<td>Galanin KO</td>
<td>24</td>
<td>10.60±1.44</td>
<td>9.50±1.36</td>
<td>34.3±4.4</td>
<td>24.2±2.1</td>
</tr>
<tr>
<td>Galanin wild-type</td>
<td>22</td>
<td>7.24±0.84</td>
<td>7.95±0.91</td>
<td>33.2±5.1</td>
<td>26.1±2.4</td>
</tr>
<tr>
<td>$P$ value&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>0.05591</td>
<td>0.35669</td>
<td>0.82626</td>
<td>0.56309</td>
</tr>
<tr>
<td>GLP-1 dose 10 nmol/kg</td>
<td>24</td>
<td>1.14±0.09</td>
<td>2.43±0.04</td>
<td>24.0±3.3</td>
<td>11.4±0.5</td>
</tr>
<tr>
<td>Insulin at 0.1 U/kg</td>
<td>10</td>
<td>2.96±0.37</td>
<td>3.16±0.38</td>
<td>18.6±4.3</td>
<td>12.8±2.6</td>
</tr>
<tr>
<td>Control of GLP-1</td>
<td>23</td>
<td>3.57±0.50</td>
<td>3.70±0.29</td>
<td>12.4±1.7</td>
<td>13.4±1.0</td>
</tr>
<tr>
<td>$P$ value&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>&lt;0.0005</td>
<td>&lt;0.0007</td>
<td>0.0017</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Values are means ± SE. $CS_T$ and $CS_G$ were calculated a posteriori after estimation of parameters $\alpha$ and $\lambda$ of Eqs. 1 and 2, respectively. <sup>a</sup>Comparison between parameters obtained in mice given pituitary adenylate cyclase-activating polypeptide (PACAP) and in relative control animals; <sup>b</sup>comparison between parameters obtained in wild-type mice and in animals with galanin-deleted gene [knockout (KO)], respectively; <sup>c</sup>ANOVA (Bonferroni/Dunn) among parameters of animals given GLP-1, insulin, and glucose only (control); control vs. insulin always nonsignificant.

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different from those used in the CSI formula and in that for CSG. Therefore, the constants initially estimated from control animals (Table 2) are recommended for any further analysis of IVGTT data because, by using this simple formula, it is possible to quantify an important metabolic parameter without using sophisticated mathematical modeling and complex computer programs.

Besides $K_G$ and $S_I$, glucose effectiveness is also important for understanding glucose disappearance (10). In fact, despite the absence of insulin, injected glucose is able to disappear from blood and to be normalized, albeit at a much slower rate than in animals (1) and humans (25) with healthy $\beta$-cell function. A similar feature has also been observed in mice injected with glucose and under diazoxide and somatostatin infusion, which blocked insulin response (23). Glucose effectiveness, parameter $S_G$ in Bergman’s minimal model (9), has been defined as glucose disappearance per se and represents the combined ability of glucose to stimulate its own uptake and suppress endogenous glucose production independent of any increase of insulin; i.e., at $I_0$. Our current study also provides simple measurements of this parameter in mice, by calculating $CS_G = 8.13 - 1.99 \times CS_I + 4.21 \times (K_G/AUC_T)$, where $CS_I$ is known from the previous calculation, and $K_G$ and $AUC_T$ are directly computed on the IVGTT data. Parameter $CS_G$ is glucose effectiveness, which accounts for the different levels of $I_0$. In this way, it represents the net contribution of glucose per se to its own disappearance, as adopted in humans to segregate the relative contributions of zero-insulin and $I_0$ effectiveness to $S_G$ (15). In addition, the units of $CS_G$ are the same as for CSI; therefore, it is easier to quantify in the same scale the effects on glucose disappearance of both insulin-dependent and insulin-independent processes (23). Glucose effectiveness has been evaluated to characterize glucose intolerance in humans (e.g., Ref. 4) and mice (e.g., Ref. 7); however, some questions have arisen on the validity of the performance of the minimal model in estimating this parameter. Since the very first study on glucose effectiveness (1), this parameter has been widely investigated without having reached a validation against an independent and accepted measurement (such as the glucose infusion in the clamp for SI). Therefore, unlike $S_I$, which is a robust index independent of the prevailing levels of insulin concentration (24), it is not yet known whether $S_G$ is equivalent to the real dynamic insulin-independent glucose disappearance. This is a reason why in this study we propose the use of $S_G/I_0$ instead of $S_G$ alone. In addition, it has been proposed that the minimal model $S_G$ may be overestimated in dogs and humans in the presence of elevated levels of dynamic insulin (12, 24). This is likely true also in mice and would explain the discrepancy between $CS_G$ and $S_G/I_0$ found with GLP-1 (Table 4), which induced a markedly elevated insulinemia. Because of the above considerations, in this study, we focused more on the equivalence of $CS_I$ with $S_I$, rather than between $CS_G$ and $S_G/I_0$; however, we feel, as well, confident that the minimal model-independent calculation of $CS_G$ may be more reliable than the model-estimated $S_G$, since it follows a specific rationale that was demonstrated valid, at least in mice (23). Thus, keeping in mind the above caveats, we suggest a reliable employment of $CS_G$ when a study is carried out to evaluate differences in a same group: for instance, before and after a specific treatment.

The validation of these new formulas results from several different investigations where $CS_I$ and $CS_G$ were estimated in mice. Although the physiological concepts arisen from these results have been, for the most part, already reported elsewhere, we briefly show that the simple calculations confirm the conclusion previously obtained. It was shown that PACAP38 and PACAP27 both reduced $CS_I$ with similar potency, without having any significant effect on $S_G$; this confirms previous observations obtained with the minimal modeling that showed that the large insulinotropic action of PACAP is compensated by reduced insulin sensitivity (11). Also, results obtained with GLP-1, which has a powerful stimulatory action of insulin secretion, were confirmed: high-dose GLP-1 administration reduces $CS_I$ (5). The reduction of $CS_I$ after GLP-1 seems in contrast with the long-term effect of the peptide to improve insulin sensitivity when used as a treatment in type 2 diabetes (28). However, the reduction of $CS_I$ might be explained by the acute high dose of 10 nmol/kg used in mice, which extensively stimulates insulin secretion. In humans, the lower dose of 75 pmol·kg$^{-1}$·h$^{-1}$ did not appreciably affect the peripheral uptake of glucose in studies on glucose turnover using the labeled glucose technique (14), and this is confirmed by $CS_I$, comparable to that of the animals that received only glucose.

The results on the various experiments highlighted another important characteristic of the simplified formulas, which may be seen as an advantage in the parameter estimation. The standard error of the outcomes from the simple formulas is in general lower than that of the minimal model parameters, showing higher accuracy of the variable. However, it is necessary to point out that the precision of the estimate: i.e., the extent to which it is similar to the “true” value, may not always be as strict. In fact, $CS_I$ tends to be higher with PACAP and GLP-1, while in control and wild-type mice the difference is minimal. This aspect deserves some further comments. The minimal model does estimate $S_I$ and $S_G$ simultaneously, balancing the contribution of the two parameters to find the best fit of the glucose data (21). The simplified formulas introduced here, instead, calculate the two parameters in sequence and the natural balance between $CS_I$ and $CS_G$ may be loose. This seems to yield a tendency for $CS_I$ to overestimate insulin sensitivity, as it can be also detected by observing Fig. 2, especially in situations of elevated insulin resistance, such as that induced by PACAP and GLP-1 (5, 11). It is worth noting, however, that also $SI$ exhibits imprecision for low values of insulin sensitivity (18); therefore, both parameters should be used with particular caution in the situation of high insulin resistance. This may be viewed as a limitation of the new method; however, it must be taken into account that all the physiological conclusions are maintained with the simplified parameters. In the galanin knockout mice, $CS_I$ is, on the contrary, slightly lower than $S_I$ but the difference, in this case, is meaningless since both parameters are in the upper limits of insulin sensitivity.

The IVGTT with seven samples has the advantage to be an experimental procedure rather simple to perform even in small animals. The glucose injection largely perturbs the state of the animals and allows the detection of possible failing mechanisms of the dynamic metabolic control. Despite that it does not supply information on the incretin
effect on the β-cells, the IVGTT provides also a measure of the directly glucose-stimulated insulin secretion. Although this was not systematically examined in this study, parameters α and λ of Table 2 may depend on the particular mouse species, laboratory procedures, assays, and precision of the statistical software. Every laboratory could derive its own set of α- and A-parameters in a group (not necessarily as numerous as ours) of their animals, by simply following the reasoning described here. A preliminary set of IVGTT experiments should be carried out and fully analyzed to tailor α- and A-parameters to their species, experimental group, or measurement assay. In our lab, for instance, we found out that already 40 experiments were sufficient for a reliable estimation of those coefficients. Nonetheless, given the similar parameters obtained from the additional analyses performed, starting from experiments with PACAP, GLP-1, and galanin, instead of from control animals, we are confident that the “general” parameters suggested here (Table 2) are applicable to different strains and in the wide range of glucose tolerance.

**Perspectives and Significance**

Simple formulas are introduced for calculating insulin sensitivity and glucose effectiveness in mice after IVGTT with a few samples and without the requirement of performing data analysis with complex mathematical modeling. The formulas revealed to be valid independent of the mouse strain. Mice are largely and increasingly used in metabolic studies. A simple method to assess insulin sensitivity and secretion adds further value to this aspect. The formulas have been extensively validated by data analysis with complex mathematical modeling. The simple formulas revealed to be valid independent of the mouse strain. Mice are largely and increasingly used in metabolic studies.

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**REFERENCES**


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