Evaluation of nonlinear regression approaches to estimation of insulin sensitivity by the minimal model with reference to Bayesian hierarchical analysis

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Evaluation of nonlinear regression approaches to estimation of insulin sensitivity by the minimal model with reference to Bayesian hierarchical analysis. Am J Physiol Endocrinol Metab 291: E167–E174, 2006. First published February 14, 2006; doi:10.1152/ajpendo.00328.2004.—Minimal model analysis of intravenous glucose tolerance test (IVGTT) glucose and insulin concentrations offers a validated approach to measuring insulin sensitivity, but model identification is not always successful. Improvements may be achieved by using alternative settings in the modeling process, although results may differ according to setting, and care must be exercised in combining results. IVGTT data (12 samples, regular test) from 533 men without diabetes was modeled by the traditional nonlinear regression (NLR) approach, using five different permutations of settings. Results were evaluated with reference to the more robust Bayesian hierarchical (BH) approach to model identification and to the proportion of variance they explained in known correlates of insulin sensitivity (age, BMI, blood pressure, fasting glucose and insulin, serum triglyceride, HDL cholesterol, and uric acid concentration). BH analysis was successful in all cases. With NLR analysis, between 17 and 35 IVGTTS were associated with parameter coefficients of variation (PCVs) for minimal model parameters $S_i$ (insulin sensitivity) and $S_0$ (glucose effectiveness) of $>100\%$. Systematic use of each different approach in combination reduced this number to five. Mean (interquartile range) $S_i^{\text{NLR}}$ was then 3.14 (2.29–4.63) min$^{-1}\cdot$mg$^{-1}$kg$^{-1} \cdot$min$^{-1}$ and 2.56 (1.74–3.83) min$^{-1}\cdot$mg$^{-1}$kg$^{-1} \cdot$min$^{-1}$ for $S_i^{\text{BH}}$ (correlation 0.86, $P < 0.0001$). $S_i^{\text{NLR}}$ explained, on average, 10.6% of the variance in known correlates of insulin sensitivity, whereas $S_i^{\text{BH}}$ explained 8.5%. In a large body of data, which BH analysis demonstrated could be fully identified, use of alternative modeling settings in NLR analysis could substantially reduce the number of analyses with PCVs $>100\%$. $S_i^{\text{NLR}}$ compared favorably with $S_i^{\text{BH}}$ in the proportion of variance explained in known correlates of insulin sensitivity.

parameter estimation; population modeling; metabolic syndrome

Since its inception in 1979 (2), the minimal model of glucose disappearance has been used extensively in studies of insulin sensitivity (and insulin resistance, i.e., pathologically low levels of insulin sensitivity). In contrast to the euglycemic hyperinsulinemic clamp reference method, minimal model analysis of intravenous glucose tolerance test (IVGTT) glucose and insulin concentrations involves a relatively straightforward clinical procedure but a complex mathematical treatment of the results, which may not always be successful in returning reliable estimates of insulin sensitivity.

In minimal model methodology, both the IVGTT procedure and the mathematical modeling analysis have undergone a number of developments, but most attention has hitherto been paid to the procedure. Modifications of the IVGTT have been introduced to augment endogenous insulin secretion (26) and to enable minimal model analysis to be used where insulin secretion is absent (25), and reduced sampling schedules have been proposed and validated (7, 19). Less attention has been paid to the process whereby the minimal model measurement of insulin sensitivity, $S_i$, is derived from observed IVGTT glucose and insulin concentration profiles. This process of so-called “model identification” requires, for a given set of IVGTT data, solution of the differential equations of the minimal model. Values for the parameters of the equations quantify relationships between glucose and insulin concentrations for a given set of IVGTT data and provide a measurement of insulin sensitivity.

Traditionally, model identification has been achieved by nonlinear regression (NLR) techniques, according to which values for equation constants are derived by computational methods that involve fitting a predicted IVGTT glucose concentration profile to the observed profile (16). In the process of model identification, a number of settings may be varied, each of which may affect model identifiability and parameter values. Some of these effects have been the subject of a previous study (13), in which different approaches to model identification were compared for their ability to distinguish groups known to differ in their insulin sensitivity and, in a subsample, for their comparability with values from the euglycemic hyperinsulinemic clamp. Further insight into the effect of changing settings on model identifiability and parameter values could be gained using very large samples, which would be expected to encompass a greater variety of IVGTT glucose and insulin profiles. In such studies, use of the euglycemic hyperinsulinemic clamp as a reference is impractical. Bayesian approaches to minimal model identification may, nevertheless, allow for reference estimates of identifiability and precision (8, 9, 18). According to the Bayesian approach, a priori knowledge of the properties of insulin sensitivity is incorporated into the identification process. We (1) have previously described an improvement in the success rate of minimal model analyses, using a Bayesian hierarchical (BH) approach in which individual values of $S_i$ were derived with reference to the distribution of values in the study group as a whole. This hierarchical, population approach...
METHODS

Design. The HDDRISC Study is a cohort study of metabolic risk factors for the development of coronary heart disease and diabetes mellitus [described in detail elsewhere (11, 12)]. The study derives from a company health program in which, from 1971 to 2000, participants received a range of metabolic, clinical, and laboratory measurements. Participants in the program were invited to undergo an IVGTT. The present analysis concerns the 533 white males who underwent an IVGTT with measurement of plasma glucose and insulin concentrations (tests were carried out between 1987 and 1995), who had a fasting plasma glucose (FPG) <7.0 mmol/l and were not found to have any abnormality of glucose metabolism. Full, informed consent for the study was obtained in each case and local ethics committee approval was given.

Procedures. Participants were instructed to consume >200 g/day carbohydrate in their diet for the previous 3 days as preparation for the IVGTT, to have fasted overnight (>12 h), and to have taken only water and refrained from cigarette smoking on the morning of their test. Height and weight were measured, and a clinical history was taken, including details of smoking, alcohol, and exercise habits. After resting for 15 min in a semirecumbent position, systolic and diastolic blood pressures were measured by a cuff method with a mercury sphygmomanometer. An indwelling cannula was inserted into an antecubital vein in each arm. With the volunteer semirecumbent, blood samples were taken for fasting plasma and serum measurements. All samples were kept on ice, plasma or serum was separated within 1 h of being taken, and routine biochemical variables were measured on the same day. Plasma samples for measurement of insulin were frozen immediately. An intravenous glucose injection (0.5 g glucose/kg as a 50% wt/vol solution of dextrose, given over 3 min) via the cannula in the opposite arm to the sampling arm. Blood samples (10 ml) were then taken at 3, 5, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 min for measurement of blood sugar and insulin.

Laboratory measurements. Plasma glucose concentrations were measured on the day of sampling by a glucose oxidase procedure (21). Plasma insulin concentrations were measured on samples stored at −20°C by a double antibody radioimmunooassay using materials supplied by Guildhay, Surrey, UK. Quality control was monitored, with pooled plasmas kept in frozen storage and commercially available lyophilized sera and by participation in national schemes [National External Quality Assurance Scheme (NEQAS) and Radioimmunoassay Quality Assurance Scheme (RIQAS)]. Particular attention was given to maintaining long-term continuity of measurement with replicate assay of previously analyzed samples held in frozen storage, extensive comparisons when there was any change in assay methodology, and examination of long-term measurement variation for any signs of assay drift. Within- and between-batch CVs ranged between 2 and 3% (plasma glucose) and 4 and 6% (plasma insulin).

Serum triglycerides were measured by fully enzymatic methods. HDL cholesterol was measured after sequential precipitation with heparin and manganese ions (24). Within- and between-batch assay CVs were 1–2% for serum triglycerides and 2–4% for HDL cholesterol. Participants also received a liver function test profile including measurement of serum uric acid concentrations.

IVGTT modeling analysis. The present study employed a regular IVGTT protocol (i.e., without exogenous administration of insulin or tolbutamide), but with a relatively high glucose dose (0.5 g/kg). In our experience, among people without diabetes, this provides for a high rate of model identification and good correlation with the reference euglycemic clamp technique (r = 0.92) despite the absence of augmentation of insulin concentrations by tolbutamide or insulin injection (20, 23). The equations of the minimal model are as follows:

\[ \frac{dG}{dt} = -p_{G_1} G - X G + p_{G_0} \]
\[ \text{(initial conditions } G_0 = G_0 \text{ and } X_0 = 0) \]

where \( G \) is the plasma glucose concentration at time \( t \), \( I \) the plasma insulin concentration at time \( t \), \( G_0 \) the basal glucose, and \( I_0 \) the basal insulin concentrations; \( p_1, p_2, \) and \( p_3 \) are the unknown parameters of the equation and \( G_0 \) the glucose concentration at time 0 that would have obtained had there been instantaneous injection and mixing of the intravenous glucose load. Parameter values were constrained to be not less than zero, since it was found that, otherwise, the time for a modeling analysis to reach completion was unacceptably lengthened. As described previously (13), the only difference that use of this constraint made was that, in place of negative parameter estimates, parameter estimates of zero or exceptionally high PCVs obtained when the constraint was used. The basal glucose concentration \( G_0 \) was taken as the mean of the fasting and 180-min values (5) and the basal insulin concentration was taken as the minimum encountered during the IVGTT. An identification returning PCV SI and PCV SI < 100% was considered acceptable.

Five alternative NLR analyses were compared in the present study. These differed in their settings as follows:

1) \( p_2 \) and \( p_3 \) invariant; curve fitting to commence at the 10-min sample; Eqs. 1 and 2 were used for model identification.

2) \( p_2 \) and \( p_3 \) invariant; curve fitting to commence at the 10-min sample; Eqs. 1 and 3 used for model identification (i.e., \( p_3 = S_0 \)).

3) \( p_2 \) and \( p_3 \) invariant; curve fitting to commence at the 7-min sample; Eqs. 1 and 2 used for model identification.

4) \( p_2 \) and \( p_3 \) invariant; curve fitting to commence at the 7-min sample; Eqs. 1 and 3 used for model identification (i.e., \( p_3 = S_0 \)).

5) as for analysis 1, but with identifications returning PCV SI or PCV SI ≤ 100% substituted by any identification from analysis 2 with PCV SI or PCV SI < 100% then by any identification...
from analysis 3 and then analysis 4 with PCV $S_{\text{NLR}}^1$ and PCV $S_{\text{NLR}}^1 < 100%$; and then by any IVGTT that could be identified with PCV $S_{\text{NLR}}^1$ and PCV $S_{\text{NLR}}^1 < 100%$ by changing the initial parameter estimates for $p_2$ and $p_3$. Analysis 5 was designed to maximize the success rate of the NLR approach by employing the full range of possibilities for changing the model identification settings.

NLR results for analysis 5 were, therefore, predominantly derived from analyses 1–4. To maximize the success rate of NLR analysis in analyses 1–4, four different implementations of the minimal model were applied uniformly in each analysis, and the optimum results from these four implementations were selected to give the final results for analyses 1–4. The four implementations have been explored previously and are described in Ref. 13. The four implementations were carried out as follows: 1) with an imputed mean at 360 min, the imputed glucose concentration being taken as the mean of the fasting and the 180-min glucose concentrations and the insulin concentration as the minimum insulin concentration measured during the procedure; 2) with overweighting of the 10-min IVGTT glucose concentration, which has the effect of forcing the fitted glucose concentration profile through the 10-min glucose measurement (16); 3) with both imputation and overweighting; and 4) with neither imputation nor overweighting. For each IVGTT, results from the identification returning the lowest PCV for $S_{\text{NLR}}^1$ were selected to comprise a listing of best identifications. If the accompanying PCV for SG were obtained as an integral part of the analysis. Individual parameters were selected. In this way, the number of successful identifications for each analysis in analyses 1–4 was maximized.

It should be noted that the value of GB with use of an imputed measure is the mean of the fasting and final IVGTT glucose concentrations, whereas without imputation it is simply the final IVGTT glucose concentration. Differences in GB then result in small but consistent differences in parameter values, as previously described (13) using the present dataset in the context of analysis 1 above, $S_1 = 3.21$ and $3.16$ min $^{-1} \cdot$ mU $^{-1} \cdot$ l$^{-1} \cdot$ s$^{-1}$ for implementations 1 and 3 with imputation, and mean $S_1 = 3.62$ and $3.49$ min $^{-1} \cdot$ mU $^{-1} \cdot$ l$^{-1} \cdot$ s$^{-1}$ for implementations 2 and 4 without imputation. Any loss of precision in combining data from the four implementations was considered to outweigh the gain derived by maximizing the number of successful identifications. In background analyses, an alternative way of combining the results from the four implementations was explored, whereby successful identifications from implementation 1 were given priority, then successful identifications from implementation 3, and then implementations 2 and 4. This made no difference to the findings.

Bayesian hierarchical analysis. Bayesian hierarchical (BH) analysis was employed to identify the minimal model for each of the 533 IVGTTs as described previously (1). In brief, “vague” prior distributions were adopted. These prior distributions were, in effect, updated from the IVGTT glucose and insulin measurements using the Bayes theorem to provide individual posterior distributions. These posterior distributions are equivalent to the parameter point estimates derived by NLR analysis in that they embody the results of the identification procedure. However, the posterior distributions are information rich and can be used to determine the precision of each parameter estimate by using a measurement of dispersion such as the CV of the distribution. Because Bayesian analysis demands that the form of underlying distributions be specified, we assumed that $S^1_{\text{BH}}$ and $S^1_{\text{BH}}$ are log-normally distributed. This has the effect of guaranteeing their nonnegativity and, hence, physiological plausibility. Parameterization was according to Eqs. 1 and 3 above, with fixed initial estimates for all parameters. Initial parameter values for $p_1$, $G_0$, $p_2$, and $S_1$ were 0.0177, 388, 0.002, and 0.0000096, respectively. The basal glucose and insulin concentrations were taken as the 180-min glucose values.

Individual estimates of $S^1_{\text{BH}}$ and $S^1_{\text{BH}}$ were taken as the medians of the posterior distributions and the precision of their estimates as the CV of the posterior distributions. Bayesian analysis is such that population parameters (mean, credible interval, interquartile range) are obtained as an integral part of the analysis. Individual parameters estimated with higher precision then contribute with greater weight to the population characteristics.

In background analyses, in addition to the BH, population-based approach described above, we also derived estimates of $S_1$ and $S_2$ on an individual basis using the Bayesian Markov chain Monte Carlo (MCMC) estimation. Parameterization according to Eqs. 1 and 3 (i.e., with $p_1 = S_1$) was unsuccessful, either with a uniform prior for each parameter on the interval $[10^{-7} - 1]$ or with an improper nonnegative uniform prior (prior values constant over nonnegative values). Parameterization according to Eqs. 1 and 2 above enabled parameter estimation, providing that the prior rather than improper uniform prior was assumed. However, 95% confidence interval of PCV $S_{\text{MC}}^{\text{MC}}$ and PCV $S_{\text{MC}}^{\text{MC}}$ was from 81 to 103% and from 235 to 256%, respectively. Therefore, at least with our reduced sample schedule, the Bayesian individual analysis did not achieve sufficient precision for reliable parameter estimation.

We employed the public domain WinBUGS program (14) extended by a purpose-made module implementing the numerical solution of the minimal model equations. The WinBUGS program adopted the Metropolis-Hastings algorithm (15) to calculate a single chain with 26,000 samples (with thinning of 4), from which the first 6,000 samples were discarded and the remaining 20,000 samples were used for further analysis. Convergence criteria of the chain were tested using the Geweke method and the Raferty-Lewis method implemented in the CODA package (3). The calculations were performed on a PC running Microsoft Windows NT operating system, with 512 MB RAM and a single 650-MHz Pentium processor. The generation of the chain with 26,000 samples took ~12 h.

Data analysis. Statistical analyses were carried out using the STATA 8 statistical package (Stata, College Station, TX). For subsequent parametric statistical analysis, measurements were log transformed, as appropriate, to normalize their distributions. The IVGTT insulin area under the curve above the fasting level (IVGTT insulin response) was calculated by the trapezium rule, and quintile limits for the IVGTT insulin response were derived. For each analysis, the observed compared with the expected number of model identifications associated with PCV $S_{\text{NLR}}^{\text{NLR}}$ and PCV $S_{\text{NLR}}^{\text{NLR}}$ ≥ 100% or outright modeling failure in bottom (suggesting impaired B-cell function) and top (suggesting low insulin sensitivity) quintiles were derived. Relationships between values of $S_{\text{NLR}}^{\text{NLR}}$ and $S_{\text{BH}}^{\text{BH}}$, and of $S_{\text{NLR}}^{\text{NLR}}$ and $S_{\text{BH}}^{\text{BH}}$ were explored using linear regression and Pearson correlation analysis, and mean values were compared by paired t-test. In accord with Bland and Altman (4), differences between $S_{\text{NLR}}^{\text{NLR}}$ and $S_{\text{BH}}^{\text{BH}}$, and between $S_{\text{NLR}}^{\text{NLR}}$ and $S_{\text{BH}}^{\text{BH}}$ were evaluated in relation to the means of $S_{\text{NLR}}^{\text{NLR}}$ and $S_{\text{BH}}^{\text{BH}}$ of $S_{\text{NLR}}^{\text{NLR}}$ and $S_{\text{BH}}^{\text{BH}}$. Univariate linear regression analyses of age, body mass index (BMI), diastolic and systolic blood pressures, fasting plasma glucose and insulin concentrations, and fasting serum triglyceride, HDL cholesterol, and uric acid concentrations with SI and SG were undertaken. The percentage of the variance in these measurements explained by $S_{\text{NLR}}^{\text{NLR}}$ and $S_{\text{NLR}}^{\text{NLR}}$ was taken as the regression analysis $R^2$ value, adjusted for the number of degrees of freedom.

RESULTS

BH analysis returned no identification with PCV $S_1$ or $S_2$ > 100%. For the four alternative general implementations of the NLR analysis (Table 1), the number of identifications returning PCV $S_1$ or $S_2$ > 100% ranged between 17 (analysis 1: minimal model Eqs. 1 and 2, 10-min mixing phase, initial estimates for $p_2$ and $p_3$ invariance) and 35 (analysis 2: minimal model Eqs. 1 and 3, 10-min mixing phase, initial estimates for $p_2$ and $p_3$ invariance). For NLR analyses 1–4, the proportion of identifications with PCV $S_1$ or $S_2$ > 100% among IVGTTs with an insulin response in the lowest quintile was approximately doubled for each analysis (Table 1), although not all such identifications were associated with a low IVGTT insulin
Table 1. Alternative approaches to minimal model identification by NLR

<table>
<thead>
<tr>
<th>NLR Minimal Model Identification</th>
<th>Failed Fits: Number Observed/Number Expected†</th>
<th>Regression Coefficient (95% CI) with SGBH (log data)</th>
<th>R Value</th>
<th>Regression Coefficient (95% CI) with SGBH (log data)</th>
<th>R Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Eqs. 1 and 2/10-min mixing phase/initial values for p2 and p3 invariant</td>
<td>3 14</td>
<td>17 5/3</td>
<td>1/3</td>
<td>0.834 (0.791–0.877)</td>
<td>0.86</td>
</tr>
<tr>
<td>2  Eqs. 1 and 4 (i.e., p3 = S0/10-min mixing phase/initial values for p2 and p3 invariant</td>
<td>14 24</td>
<td>35 13/7</td>
<td>1/7</td>
<td>0.848 (0.804–0.892)</td>
<td>0.86</td>
</tr>
<tr>
<td>3  Eqs. 1 and 2/7-min mixing phase/initial values for p2 and p3 invariant</td>
<td>8 15</td>
<td>23 10/5</td>
<td>0/5</td>
<td>0.839 (0.795–0.882)</td>
<td>0.86</td>
</tr>
<tr>
<td>4  Eqs. 1 and 2 (i.e., p3 = S0/7-min mixing phase/initial values for p2 and p3 invariant</td>
<td>17 13</td>
<td>29 12/5</td>
<td>2/5</td>
<td>0.836 (0.791–0.881)</td>
<td>0.85</td>
</tr>
<tr>
<td>5 As for 1, but with identifications with PCV &gt;100% substituted by 4, then 3, then 2 and then by identifications with different initial values for p2 and p3 to minimize PCV &gt;100%</td>
<td>1 4</td>
<td>5 0/1</td>
<td>0/1</td>
<td>0.831 (0.789–0.874)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

S0, insulin sensitivity; S0G, glucose effectiveness; BH, Bayesian hierarchical approach; NLR, nonlinear regression approach; PCV, parameter coefficient of variation; CI, confidence interval. Numbers of failures (i.e., PCV S0 or S0G >100%) for intravenous glucose tolerance test insulin responses associated with failures and comparison of S0 and S0G with results from BH identification. Alternative approaches 1–5 are as listed in METHODS. Regression coefficients and R values are derived for cases with PCV S0 and S0G <100%. All regression coefficients and R values were significant at P < 0.001. †Number expected is derived by assuming that model failures will be evenly distributed throughout the 5 quintiles of S0 values.

response. The proportion of identifications with PCV S0 or S0G >100% among IVGTTs with an insulin response in the highest quintile was appreciably lower than expected.

Of the 17 IVGTTs that could not be successfully identified using our default protocol (analysis 1), nine were successfully identified by making S0 a parameter of the equations or by including the 7-min IVGTT glucose and insulin measurements or both. Of the remaining eight, three were successfully modeled by changing initial estimates for p3 or p2. The combined data from this sequence of analyses then resulted for analysis 5. Individual examination of the five cases that could not be successfully modeled in analysis 5 revealed that, for one, PCV S0 was 101%. This case was associated with an IVGTT glucose elimination constant, k, in the bottom (first) quintile and an IVGTT insulin response in the fourth quintile. Consistent with these features, S0\text{NLR} was low (0.66 min\textsuperscript{-1·}μU\textsuperscript{-1}·10\textsuperscript{-5}), although this was not confirmed by S0\text{BH} (2.12 min\textsuperscript{-1·}μU\textsuperscript{-1}·10\textsuperscript{-4}). For the other four cases, PCV S0G ranged between 108 and 286%. Each of these cases was similarly distinguished by having values for S0\text{NLR} in the top quintile, which was supported by similar values for S0\text{BH}. The difference between S0\text{NLR} and S0\text{BH} for the case with PCV S0\text{NLR} = 101% did not lie outside 2 SD of the mean difference, neither did the differences between S0\text{NLR} and S0\text{BH} for the four cases with PCV S0\text{G} between 108 and 286%. Further insight into these NLR modeling failures was gained by evaluating the Markov chain Monte Carlo individual Bayesian analysis (results not shown). This showed that the failures of NLR are primarily related to low precision of S0 estimates caused by the high variance associated with the marginalized likelihood function. Furthermore, in accord with Pillonetto et al. (17), we noted a complex non-Gaussian joint likelihood. Pillonetto et al. concluded that S0 and p2 are at risk of poor numeric identifiability, especially as NLR represents the joint likelihood by the joint mode. We concur that the Bayesian analysis provides more robust estimates such as the median of the marginalized likelihood function or other statistical measurements obtained by integrating over the parameter space. This is particularly beneficial in cases of high correlation within or highly complex joint likelihood function as experienced in our data set. Unlike Pillonetto et al., we did not use informative prior for S0 or any other parameter; thus the computational improvements cannot be attributed to the use of informative prior.

The distribution of individual parameter values was considered in terms of the individual parameter value 95% confidence intervals. As expected, those parameters showing PCV >100% with NLR analysis were associated with broad confidence intervals, but in each case confidence intervals from NLR and BH analyses overlapped. In the dataset as a whole, 95% confidence intervals from NLR and BH analyses did not overlap for 44 estimates of S0 and 11 estimates of S0G. These estimates were generally not associated with high-parameter CVs.

Very similar regression coefficients of S0\text{NLR} with S0\text{BH} were obtained (range 0.831–0.848; Table 1) and for S0\text{G} from NLR with S0\text{BH} (range 1.043–1.166). In accord with these coefficients, geometric mean values for S0\text{NLR} were very similar (Table 2) but, on paired testing, significantly higher than S0\text{BH} (P < 0.001). Geometric mean values for S0\text{G} were also very similar but significantly lower than S0\text{BH} (P < 0.001).

Trends in the differences between S0\text{NLR} and S0\text{BH} and between S0\text{NLR} and S0\text{BH} with the mean values of S0\text{NLR} and S0 were illustrated by the Bland-Altman difference plots in Fig. 1. These plots revealed a tendency for S0\text{NLR} to be somewhat higher than S0\text{BH} at intermediate values of S0 but lower at higher values. For S0G there was a tendency for S0\text{NLR} to be higher than S0\text{BH} at high values of S0G. Mean
PCVs for SI\textsuperscript{BH} and SI\textsuperscript{NLR 5} were 13.7% (range 4–60%) and 21.5% (range 2–101%), respectively. For SG, the equivalent figures were 12.3% (range 5–22%) and 39.2% (range 3–286%). Scatters of SI\textsuperscript{NLR 5} vs. SI\textsuperscript{BH} and of SG\textsuperscript{NLR 5} vs. SG\textsuperscript{BH} are shown in Fig. 2.

The five different NLR approaches returned very similar mean estimates for the glucose volume of distribution (0.133 to 0.136 l/kg body wt) and very similar mean values for p\textsubscript{2} (0.025 to 0.027). The mean glucose volume of distribution and mean p\textsubscript{2} for the Bayesian analysis were 0.172 l/kg body wt and 0.029, respectively. BH analysis, therefore, returned a very similar mean value for p\textsubscript{2} but a 28% higher glucose volume of distribution.

The percentages of the variance (i.e., the regression analysis $R^2$ value, adjusted for the number of degrees of freedom) explained by SI\textsuperscript{NLR 5} (all cases included) and SI\textsuperscript{BH} in age, BMI, diastolic and systolic blood pressures, fasting plasma glucose and insulin concentrations, and fasting serum triglyceride, HDL cholesterol, and uric acid concentrations are shown in Table 3. A greater percentage of the variance for each correlate of insulin sensitivity was explained by SI\textsuperscript{NLR 5} than by SI\textsuperscript{BH} for all variables except fasting plasma glucose.

Table 2. Geometric means and interquartile ranges for SI\textsubscript{n} and SG\textsubscript{g}, derived by NLR and BH methods for cases with PCV SI\textsubscript{n} and SG\textsubscript{g} <100%.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Interquartile Range</th>
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<tbody>
<tr>
<td>$S_{iBH}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-4}$</td>
<td>2.56</td>
<td>1.74–3.83</td>
</tr>
<tr>
<td>$S_{iNLR 1}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-4}$</td>
<td>3.12</td>
<td>2.28–4.60</td>
</tr>
<tr>
<td>$S_{iNLR 2}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-4}$</td>
<td>3.10</td>
<td>2.25–4.41</td>
</tr>
<tr>
<td>$S_{iNLR 3}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-4}$</td>
<td>3.07</td>
<td>2.21–4.48</td>
</tr>
<tr>
<td>$S_{iNLR 4}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-4}$</td>
<td>3.12</td>
<td>2.24–4.53</td>
</tr>
<tr>
<td>$S_{iNLR 5}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-4}$</td>
<td>3.14</td>
<td>2.29–4.63</td>
</tr>
<tr>
<td>$S_{gBH}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-2}$</td>
<td>1.74</td>
<td>1.35–2.21</td>
</tr>
<tr>
<td>$S_{gNLR 1}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-2}$</td>
<td>1.51</td>
<td>1.11–2.05</td>
</tr>
<tr>
<td>$S_{gNLR 2}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-2}$</td>
<td>1.52</td>
<td>1.10–2.05</td>
</tr>
<tr>
<td>$S_{gNLR 3}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-2}$</td>
<td>1.59</td>
<td>1.20–2.14</td>
</tr>
<tr>
<td>$S_{gNLR 4}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-2}$</td>
<td>1.61</td>
<td>1.20–2.16</td>
</tr>
<tr>
<td>$S_{gNLR 5}$ min\textsuperscript{-1}mU\textsuperscript{-1}L $\times 10^{-2}$</td>
<td>1.52</td>
<td>1.11–2.08</td>
</tr>
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NLR 1–5 refers to analyses 1–5 (see METHODS and Table 1).

Fig. 1. Bland-Altman plots illustrating trends in the magnitude of individual differences between $S_{iNLR 1}$ and $S_{iBH}$ with mean $S_{i}$ and of individual differences between $S_{gNLR 1}$ and $S_{gBH}$ with mean $S_{g}$, SI, insulin sensitivity; SG, glucose effectiveness; BH, Bayesian hierarchical analysis; NLR, nonlinear regression analysis; NLR 5, NLR analysis 5 (see IVGTT modeling analysis). Plus and minus 2 SD limits of differences are shown.

Fig. 2. Scatterplots of the $S_{iNLR 5}$ vs. $S_{iBH}$ and of $S_{gNLR 5}$ vs. $S_{gBH}$. Dotted line, line of equality; continuous line, fitted linear regression line.
Table 3. Percentages of variance (from univariate regression $R^2$ values, adjusted for no. of degrees of freedom) in correlates of $S_I$ explained by $S_I^{NLR}$ and $S_I^{BH}$ with regression line slopes and 95% CI of the slope (analysis includes 1 case from NLR analysis 5 with PCV $S_I = 101\%$ and 5 cases with PCV $S_G$ between 108 and 286%)

<table>
<thead>
<tr>
<th></th>
<th>$S_I^{NLR}$ Adjusted $R^2$, %</th>
<th>$S_I^{NLR}$ 5 Regression Line Slope and 95% CI</th>
<th>$S_I^{BH}$ Adjusted $R^2$, %</th>
<th>$S_I^{BH}$ Regression Line Slope and 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.8</td>
<td>$-0.0072 (-0.0118, -0.0026)$</td>
<td>1.7</td>
<td>$-0.0073 (-0.0120, -0.0026)$</td>
</tr>
<tr>
<td>Body mass index</td>
<td>20.3</td>
<td>$-0.0879 (-0.1029, -0.0730)$</td>
<td>17.9</td>
<td>$-0.0854 (-0.1010, -0.0698)$</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>6.8</td>
<td>$-0.0085 (-0.0112, -0.0058)$</td>
<td>5.3</td>
<td>$-0.0078 (-0.0106, -0.0049)$</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>6.7</td>
<td>$-0.0140 (-0.0186, -0.0096)$</td>
<td>5.0</td>
<td>$-0.0125 (-0.0172, -0.0078)$</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>3.4</td>
<td>$-0.0129 (-0.0188, -0.0070)$</td>
<td>4.0</td>
<td>$-0.0140 (-0.0203, -0.0083)$</td>
</tr>
<tr>
<td>Fasting plasma insulin</td>
<td>20.4</td>
<td>$-0.2917 (-0.3412, -0.2422)$</td>
<td>15.7</td>
<td>$-0.2658 (-0.3185, -0.2131)$</td>
</tr>
<tr>
<td>Fasting serum triglycerides</td>
<td>16.9</td>
<td>$-0.4098 (-0.4879, -0.3317)$</td>
<td>13.7</td>
<td>$-0.3824 (-0.4643, -0.3005)$</td>
</tr>
<tr>
<td>Serum HDL cholesterol</td>
<td>7.5</td>
<td>0.5263 (0.3673, 0.6853)</td>
<td>4.3</td>
<td>0.4169 (0.2496, 0.5843)</td>
</tr>
<tr>
<td>Serum uric acid</td>
<td>9.7</td>
<td>$-0.0022 (-0.0028, -0.0016)$</td>
<td>8.7</td>
<td>$-0.0022 (-0.0028, -0.0016)$</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Identification of the minimal model of glucose disappearance for the 533 reduced sampling schedule IVGTTs considered in the present analysis using a BH procedure was associated with no model failure or any identifications associated with PCV $S_I$ or $S_G > 100\%$. This demonstrates that all IVGTTs included in the present study were essentially identifiable within conventional limits of precision. By use of five different implementations for model identification (which nevertheless agreed well in the values for $S_I$ and $S_G$ that they returned), the NLR approach was also able to successfully identify all IVGTTs, albeit with five cases for which PCV $S_I$ or $S_G$ was greater than the conventional upper limit of 100%. NLR estimates of insulin sensitivity for these five cases may, nevertheless, be valid, since, in each case, results were appropriate for the IVGTT glucose profiles and insulin responses and were, in four of the five cases, supported by the Bayesian estimates. It is noteworthy that the four cases with PCV $S_G > 100\%$ were each associated with very high insulin sensitivity, suggesting that there may be some loss in precision of estimation of $S_G$ when IVGTT glucose concentrations are falling rapidly due to insulin action rather than glucose effectiveness. Conventionally, these cases would have been excluded, but it could be argued that this would have introduced a bias into the data toward lower insulin sensitivities. Identifications with PCVs somewhat greater than 100% might therefore be considered for inclusion in minimal model studies, particularly if there is additional support for the estimates of $S_I$ or $S_G$ obtained. Our analysis of this large dataset indicates that, in the absence of diabetes, IVGTTs with such intractably high PCVs only occur in about 1% of cases. Moreover, inclusion of the 5 cases with PCVs > 100% made no difference to the percentage of variance in known correlates of insulin sensitivity explained by $S_I^{NLR}$.

The IVGTT protocol that we used did not include administration of tolbutamide or insulin to augment endogenous insulin concentrations during the test but did use a higher dose than is conventionally used (0.5 vs. 0.3 g/kg). Because the minimal model simply relates the rate of decline in glucose concentrations during the IVGTT to the accompanying plasma insulin concentrations, irrespective of where that insulin is coming from, there is in principle no reason to suppose that there would be different conclusions for 0.3 g/kg IVGTT or the tolbutamide or insulin-modified versions of the IVGTT. However, the 0.3 g/kg IVGTT might still be associated with poorer identifiability on account of the lower insulin response (26). With regard to the modified protocols, parameter estimation might be affected by the form that the excursion in insulin concentrations takes, so our conclusions would have to be confirmed in a large dataset derived from one of the modified IVGTT protocols.

Direct estimation of $S_I$ as a parameter of the minimal model equation or shortening the mixing time for the glucose injection to 7 min or both were associated with a somewhat greater number of analyses with PCV $S_I$ or $S_G > 100\%$ than with estimation of $p_2$ and $p_3$ separately and a 10-min mixing time but made very little overall difference to the values of $S_I$ and $S_G$ obtained. This supports our use in analysis 5 of results from these alternative implementations to minimize the number of IVGTT modeling analyses associated with PCVs > 100%. NLR-derived estimates of $S_I$ and $S_G$ did, however, differ from the BH-derived estimates, $S_I^{NLR}$ being higher and $S_G^{NLR}$ lower than their Bayesian equivalents. This suggests that the NLR approach gives more weight to insulin-dependent and less weight to glucose-dependent processes in explaining IVGTT glucose and insulin profiles. Strong correlations nevertheless obtained between estimates of $S_I$ and $S_G$ from the two approaches, and both approaches returned values of $S_I$ that were able to explain similar proportions of the variance in known correlates of insulin sensitivity.

Because it compares different approaches to the same methodology, the present analysis cannot provide evidence of the accuracy of the estimates of $S_I$ being compared, although it is noteworthy that mean values of $S_I$ and $S_G$ differed significantly between NLR and BH approaches. In background analyses (results not shown), the difference in mean values of $S_I$ was found to be largely explained by the use of different settings for the basal glucose concentration: the 180-min value for the BH analysis and the mean of the fasting and 180-min values for the NLR analysis [use of the 180-min value in the NLR analysis led to an appreciably higher failure rate, as previously demon-
Despite lack of an accuracy reference, the substantial numbers of IVGTTs studied can allow for an operational evaluation of the validity of the $S_I$ estimates in terms of their ability to explain the variance in known correlates of insulin sensitivity. In this respect, the NLR analyses were approximately equivalent, accounting for, on average, between 9.3 and 10.6% of the variance. Different $S_{I}^{\text{NLR}}$ estimates, therefore, accounted for variation in known correlates of insulin sensitivity as effectively as estimates from the more robust BH approach, in which all IVGTTs returned PCV $S_I$ and $S_G$ <100%. It should, nevertheless, be borne in mind that NLR estimates of $S_I$ and $S_G$ can vary quite markedly according the settings used, particularly with regard to the setting for the basal glucose concentration, and caution should be exercised in combining NLR results that employ different settings.

A particular advantage of Bayesian methods is that they enable existing knowledge to be used in an analysis. This may be of value when data are limited or associated with higher degrees of uncertainty such as might obtain with reduced sampling schedules. Bayesian approaches have been extensively used in pharmacokinetic studies in which these limitations may often apply. However, they have been less used in physiological and metabolic modeling studies, which may be equally limited in the data available and the uncertainties involved in estimating unknown parameters. Only sporadic reports have appeared of the application of Bayesian techniques to minimal model of glucose of disappearance. Nevertheless, they have been used as a means of incorporating prior knowledge of glucose kinetic characteristics into a modified minimal model (8) and, in what is in effect an as a posteriori approach, have enabled derivation of minimal model parameters with reference to the population characteristics that emerge in the course of an analysis (1, 9, 18, 22). This approach has been shown to improve the precision of parameter estimation with the regular IVGTT, with both frequent (9) and reduced (22) sampling schedules. It has also been applied to the insulin-modified, frequently-sampled IVGTT as used in patients with diabetes mellitus (1), although there appears to be little advantage with the insulin-modified, frequently-sampled IVGTT as used in normoglycemic individuals (10). The Bayesian approach has also allowed for improved parameter precision and model identifiability in distinguishing the extrapancreatic effects of tolbutamide, which is used during the IVGTT to augment endogenous insulin release (18). Our BH analysis shows that the Bayesian approach may indeed have advantages over NLR in limiting identifications associated with PCV $S_I$ or $S_G$ >100%, although with systematic application of the various alternatives explored here such identifications may be minimized in NLR analysis. The mean PCV $S_I$ was somewhat lower with BH analyses than with NLR analysis, although the mean PCV $S_G$ was appreciably lower with BH analysis. In our previous studies using simulated data in healthy subjects with the insulin-modified IVGTT (10), NLR analysis provided less accurate estimates of individual $S_G$ than BH (28 vs. 20%; root mean square error as %mean). Population variability was underestimated by BH and overestimated by NLR (33 vs. 40 vs. 20; true vs. NLR vs. BH intersubject variability). This suggests that accurate estimation of population variability of $S_G$ is difficult but that BH provides more accurate estimates of individual $S_G$. Further developments in the BH approach may be considered, since, in principle, it is possible to provide more informative prior distributions with the BH analysis. This could include not only information about means and variances but also information about correlations among parameters. In the present work, we did not explore these possibilities and limited ourselves to “noninformative” prior distributions. Precise parameter estimates could be obtained with noninformative priors, and, as “informative” prior distribution influences the posterior distribution, we aimed to avoid this influence as much as possible. We acknowledge that the form of the prior distribution, i.e., the multivariate log-normal distribution, also influences the posterior estimates.

In summary, a variety of settings are available for performing NLR identification of the minimal model. Some variation may be introduced into the data by combining results from different settings, especially with respect to the use of different assignments for the basal glucose concentration $G_b$. Nevertheless, used systematically, different settings can considerably improve the overall precision of parameter estimation, such that the occurrence of parameter CVs of >100% can be reduced to less than 1% in a dataset that BH analysis has demonstrated can be fully identified with PCVs of <100%. $S_{I}^{\text{NLR}}$ compared favorably with $S_{I}^{\text{BH}}$ in the proportion of variance explained in known correlates of insulin sensitivity.

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