Population-based modeling to demonstrate extrapancreatic effects of tolbutamide

A. Rostami-Hodjegan, S. R. Peacey, E. George, S. R. Heller, and G. T. Tucker. Population-based modeling to demonstrate extrapancreatic effects of tolbutamide. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E758–E771, 1998.—Tolbutamide is used increasingly as an investigative tool in in vivo studies of the physiology of glucose tolerance. Its hypoglycemic effect in nondiabetic subjects is widely variable, reflecting possible variability in its pharmacokinetics, an insuliner- gic response, an extrapancreatic effect of the drug, or the hypoglycemic effect of insulin itself. Using population-based modeling, we have investigated the kinetics and dynamics of tolbutamide and assessed covariates in two groups of healthy subjects. The results indicate a high variability in insuliner- gic effect, measured by the area under of the curve of insulin (0–60 min), in response to tolbutamide injection (coefficient of variation = 29–96%). However, it appears that impaired insulin sensitivity is compensated by higher insulin secretion in response to tolbutamide. Thus the hypoglycemic effect of high insulin secretion is minimal in insulin-resistant sub- jects. Application of the model indicated that tolbutamide has appreciable extrapancreatic effects mediated by prolongation of the residence time of insulin in a remote effect and by enhancement of glucose effectiveness. An effect in increasing the insulin sensitivity index is also possible but could not be confirmed statistically for all groups of subjects studied. These observations may explain inconsistencies between the results of tolbutamide and insulin injection in the frequently sampled intravenous glucose tolerance test and call for further study of insulin- vs. tolbutamide-modified frequently sampled intravenous glucose tolerance tests in the assessment of the insulin sensitivity and glucose effectiveness indexes.

insulin secretion; insulin sensitivity; population pharmacokinetics-pharmacodynamics; sulfonylureas; minimal model
The possible extrapancreatic actions of tolbutamide continue to be controversial (31). Despite this controversy, the drug has been used in an improved protocol for FSIGT to assess insulin sensitivity and glucose effectiveness with greater precision (2, 38). Although not commonly acknowledged, this use of tolbutamide depends on the assumption that insulin secretion is the sole action of the drug or, at least, that any extrapancreatic effect of tolbutamide is invariant between different individuals or between different populations. This may not be so. Therefore, in analyzing our experimental data (26, 27), a particular attempt has been made to compare glucose effectiveness and insulin sensitivity in the presence and absence of tolbutamide.

Glossary

A A constant
AUC Area under the concentration (or rate)-time profile
BG Blood glucose
BG0 Fasting blood glucose
B A constant
BMI Body mass index
C Serum concentration
CE Concentration in a remote effect compartment
C50 Concentration of drug that produces half-maximum insulin secretion
CL Clearance
C/P Balance between consumption and production
ΔCInsLym Lymph insulin concentration in excess of fasting lymph insulin
ΔCinsS Serum insulin concentration in excess of fasting serum insulin
d(C/P)/dt Rate of change of C/P (glucose disposal rate)
Eh Hepatic extraction ratio
FBG Fasting blood glucose
FH Fraction of insulin in hepatic portal vein that avoids first-pass hepatic metabolism
FLym Insulin lymph-to-serum ratio at steady state
FSI Fasting serum insulin
f0 Unbound fraction of drug in serum
GEI Index of effectiveness of blood glucose in enhancing glucose consumption
I Insulin mass in respective compartment
Ins Insulin central compartment
IR Insulin resistance
ISI5 Insulin sensitivity index as measured by minimal model
ISI-Lym Index of sensitivity to effect of lymph insulin in lowering blood glucose
k0 Zero-order infusion rate
k1 First-order rate constant for transfer to compartment j from compartment i (when j = 0, this becomes an elimination rate constant)
kg A power function used to link blood glucose to insulinergetic effect of tolbutamide

METHODS

Data Base

The data base consisted of information on variable dextrose infusion, blood glucose, and serum concentrations of tolbutamide, C-peptide, and insulin (Table 1). Details of the subjects and protocols have been published elsewhere (26, 27). All the subjects were healthy nondiabetics (Table 2) with no family history of diabetes. They were asked to avoid excessive exercise and alcohol on the day before each study. The sequence of studies was randomized (unbalanced) for studies I–III and studies IV and V. Subjects fasted overnight before each study.

The measurement of arterialized blood glucose and adjustments of dextrose infusion were carried out as reported previously (26). Blood glucose was maintained at a euglycemic level for a predefined period of time (30–120 min, Table 1) and then allowed to fall gradually to a controlled hypoglycemic level (except in study I, where euglycemia was maintained throughout the experiment). Recovery from the hypo-
glycemia was achieved by an increase in the dextrose infusion and consumption of a high-calorie meal. Serum was assayed for tolbutamide (7–13 time points), insulin (7–12 time points), and C-peptide (7–8 time points), as described previously (26).

### Tolbutamide Kinetics

Serum concentrations of tolbutamide were fitted by a classical open two-compartment model with sequential unequal intravenous infusion inputs (Eq. 4 in APPENDIX) (15) using the P-Pharm population K/D program (version 1.3e, SIMED Biostatistics and Data Processing, Créteil, France). The algorithm in P-Pharm is described as being of the two-stage expectation-maximization type (24), although some consider it to be more like the iterative two-stage procedure proposed by Prevós (3).

Age, sex, weight, body surface area, serum tolbutamide binding, body mass index, lean body mass (LBM), total body fat (TBF), and type of study were investigated as covariates affecting the clearance and volume of distribution of tolbutamide. Serum drug binding was measured in the 5-min samples by ultrafiltration at 3,000 g, 37°C, for 30 min (Centrifree micropartitioning device, Amicon). Body composition (TBF and LBM) was estimated using bioelectrical impedance (model EZ 1500, Cranley Medical Electronics, Birming-

### C-Peptide Kinetics

Spline functions were used to fit serum C-peptide concentration data and to calculate individual input function curves (i.e., concentration change due to secretion per unit of time), as described by Eaton et al. (12). Population values of elimination constants for deconvolution were those reported by Polonsky et al. (29). The rate of C-peptide secretion was then calculated using the reported population value of its volume of distribution (65 ml/kg) (29). The partial area under the curve (AUCd) up to 20 min (i.e., the time that serum C-peptide achieved its maximum value) was used to compare different subjects with respect to the production of C-peptide in response to tolbutamide.

### Insulin Kinetics

With the assumption of equimolar secretion of insulin and C-peptide, the results of the analysis of C-peptide data were used as a measure of insulin secretion. To estimate the hepatic extraction ratio of insulin, individual values of insulin clearance were calculated from the insulin arms of the studies.

### Table 1. Summary of glucose clamp studies using insulin or tolbutamide

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Hypoglycemic Agent</th>
<th>No. of Subjs</th>
<th>Dosage Regimen</th>
<th>State of Glycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tolbutamide</td>
<td>8</td>
<td>1,700 mg iv over 3 min, followed by 130 mg iv from 10 min to end of study</td>
<td>Euglycemia (4.5 mmol/l) throughout study period</td>
</tr>
<tr>
<td>II</td>
<td>Tolbutamide</td>
<td>8</td>
<td>Same as study I</td>
<td>Euglycemia (4.5 mmol/l) between 0 and 70 min; hypoglycemia (2.8 mmol/l) between 90 and 120 min</td>
</tr>
<tr>
<td>III</td>
<td>Insulin</td>
<td>8</td>
<td>190, 82, 75, 70, 65, and 60 μU · min⁻¹ · m⁻² over 0–2, 2–4, 4–6, 6–8, 8–10, and 10 min to end, respectively</td>
<td>Same as study II</td>
</tr>
<tr>
<td>IV</td>
<td>Tolbutamide</td>
<td>10</td>
<td>Same as study I</td>
<td>Euglycemia (4.5 mmol/l) between 0 and 20 min; hypoglycemia (2.8 mmol/l) between 30 and 60 min</td>
</tr>
<tr>
<td>V</td>
<td>Insulin</td>
<td>10</td>
<td>Same as study III, but all doses halved</td>
<td>Same as study IV</td>
</tr>
</tbody>
</table>

Information is from Refs. 26 and 27.

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**Fasting serum insulin (FSI) and blood glucose (FBG) values are means ± SD. BSA, body surface area (weight (kg)⁰.⁴²⁵ × height (cm)⁰.⁷²⁸ × 71.84/10,000); BMI, body mass index; LBM, lean body mass; TBF, total body fat; β-cell function, 20 × FSI/(FBG – 3.5) (Ref. 33); IRI, insulin resistance index = FSI/[22.5 e⁻ ⁰.⁵FBG] (Ref. 33); Avg, average; CV, coefficient of variation; NM, not measured.**
DIX). Insulin secretion was defined at 5-min intervals from the DIX). Inasmuch as some of the subjects received tolbutamide (Eq. 16 in APPENDIX). With the assumption that tolbutamide of blood glucose on this effect.

Fig. 1. Pharmacokinetic-pharmacodynamic model used to describe biphasic insulinergic effect of tolbutamide and synergistic influence of blood glucose on this effect.

(Eq. 16 in APPENDIX). With the assumption that tolbutamide does not alter insulin clearance (25), individual extraction ratios were then calculated from integrated insulin (C-peptide) secretion between 0 and 60 min, and the respective AUC of serum insulin concentration was measured during respective tolbutamide arms of the studies (Eq. 17 in APPENDIX). Inasmuch as some of the subjects received tolbutamide on two or three occasions, it was also possible to estimate intrasubject variability in the hepatic extraction of insulin.

Model-Independent Dynamics of Tolbutamide and Insulin

C-peptide secretion was considered a measure of the insulinergic response to tolbutamide injection. Also, dextrose infusion rate was used to construct an index for glucose disposal rate (Eq. 19 in APPENDIX) and to evaluate the hypoglycemic effect of insulin in the presence and absence of the drug. Model-independent parameters (e.g., area under the curve up to 60 min) were then used to determine whether the hypoglycemic effect estimated at a given serum insulin level was comparable in the presence and absence of tolbutamide.

K/ D Modeling

Insulinergic effect of tolbutamide. In the first part of the K/D analysis, insulin secretion was modeled with respect to tolbutamide concentration and blood glucose (Eq. 1 in APPENDIX). Insulin secretion was defined at 5-min intervals from the simulations of C-peptide secretion, as explained above.

The model consisted of two kinetic compartments and an additional peripheral effect compartment that received a negligible mass of the drug (15) (Fig. 1). The individual kinetic parameters for tolbutamide, estimated as described above, were later entered as covariates into a K/D link analysis (Eq. 1 in APPENDIX).

The transfer and elimination processes were considered to be first order, as commonly assumed in classical kinetics (15). The output to the effect compartment (defined by $k_{E1}$) had no significant effect on drug concentrations in the central compartment (15). The equation describing tolbutamide concentration in the effect compartment was developed with only one unknown parameter, $K_{DI}$ (Eq. 5 in APPENDIX).

In contrast to most K/D models, which assume that the effect is exerted only by drug located in the effect compartment (15), the insulinergic effect of tolbutamide was considered to be mediated by drug in central and effect compartments producing immediate and delayed effects, respectively. The insulinergic effect in both compartments was described by Hill functions (Eq. 1 in APPENDIX).

To account for the proportional effect of hypoglycemia and hyperglycemia on the insulinergic effect of tolbutamide (28), the secretion was linked to blood glucose. Thus insulin secretion was lower during hypoglycemia in proportion to the fall in blood glucose. The time course of blood glucose was described by empirical equations that varied depending on the study (Eqs. 6–8 in APPENDIX).

Hypoglycemic effect of insulin. In the second part of the K/D analysis, the hypoglycemic effect was modeled with respect to insulin and glucose concentrations in the presence and absence of tolbutamide. The hypoglycemic effect was defined by the rate of glucose disposal [balance between consumption and production (CP), Eq. 19 in APPENDIX]. The frequency of sampling was the same as that used to monitor blood glucose. The model used the assumptions of the minimal model (4, 6): 1) glucose inhibits its own production and increases its utilization in proportion to its concentration in plasma, 2) insulin has a synergistic influence on these effects of glucose, and 3) the effect of insulin to promote the decline of glucose in plasma depends only on the concentration of insulin in a remote compartment (e.g., lymph), or, as an alternative hypothesis, the effect of insulin to promote the decline of glucose in plasma depends on the concentration of insulin in a remote compartment as well as serum insulin (Fig. 2).

The alternative hypothesis, although it investigated the importance of serum insulin relative to that of lymph insulin, served as a validation for the modeling. Thus the aim was to reaffirm the results of experimental studies that have shown negligible effects from serum insulin compared with lymph insulin. The improvement in model fitting achieved by addition of the effect from serum insulin was assessed using the Akaike information criterion (36).

Serum insulin concentrations were fitted by empirical functions that varied for the insulin and tolbutamide arms of the studies (Eqs. 9–12 in APPENDIX). In a two-stage K/D analysis, individual values of the parameters of these empirical equations were obtained (kinetics) and used as covariates in subsequent K/D link analysis. Appropriate equations were developed (by incorporating Eqs. 9–12 into Eq. 14 in APPENDIX) to describe the time course of insulin concentration in the remote (i.e., lymph; Eq. 15 in APPENDIX) compartment with two unknown parameters for the transfer rates between central and peripheral compartments (Fig. 2) that were obtained from simultaneous fitting of dynamic (glucose disposal rate) and kinetic data (blood glucose level and serum insulin concentration). Thus serum insulin concentration was linked to hypoglycemic effect via an effect compartment.
without the need to calculate actual concentrations in this compartment. The K/D link model was described by equations similar to those used in the minimal model (Eq. 3 or 4 in APPENDIX). Data for the disposal rate of glucose were used only when there was no significant difference between the insulin and corresponding tolbutamide studies with respect to the levels of counterregulatory hormones (27, 37).

By solving the K/D link model in the presence (studies I, II, and IV) and absence of tolbutamide (studies III and V), insulin sensitivity index (ISI), GEI, and $k_{OLym}$, a constant describing the elimination of insulin from lymph, were determined. The parameter $k_{OLym}$ defined the onset and duration of effect mediated by insulin in interstitial fluid.

Statistical Methods

Inter- and intraindividual variability of all parameters in the above analyses was obtained by ANOVA. A paired t-test was used to investigate differences between protocols.

For population analysis, interindividual variability was obtained directly from the computed fits. Differences between population measures in different protocols were tested using Student's t-test for inference. Student's paired t-test was used to investigate differences in a posteriori individual values obtained from different protocols.

RESULTS

Main Observations

Insulinergic effect of tolbutamide. Despite similar serum tolbutamide concentrations in the study subjects (Fig. 3A), serum C-peptide concentrations (Fig. 3B) and serum insulin concentrations (Fig. 3C) showed considerable interindividual variability during tolbutamide administration. The insulinergic effect of tolbutamide (as measured by AUC$_{C/P}$ of C-peptide) did not correlate with tolbutamide AUC on the basis of total or free serum concentrations of the drug.

Blood glucose level was stable in all subjects (Fig. 3D). However, to maintain the target level, frequent changes in dextrose infusion were required during all studies (Fig. 3E).

Despite wide variation in serum insulin concentration after tolbutamide, the hypoglycemic effect (as measured by AUC$_{C/P}$) failed to correlate with tolbutamide AUC on the basis of total or free serum concentrations of the drug.

Table 3. Model independent parameters describing glucose economy and related insulin kinetics

<table>
<thead>
<tr>
<th>Study</th>
<th>AUC$_{Ins, U}$</th>
<th>AUC$_{Ins, mg}$</th>
<th>AUC$_{C/P, AUC}$</th>
<th>AUC$_{C/P, mg}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.40 (3.64)</td>
<td>17.5 (18.0)</td>
<td>6.05 (4.72)</td>
<td>19.1 (19.0)</td>
</tr>
<tr>
<td></td>
<td>6.12 (5.97)</td>
<td>29.7 (30.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>2.06</td>
<td>21.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>29</td>
<td>29</td>
<td>20</td>
<td>35</td>
</tr>
</tbody>
</table>

All areas under the curve (AUCs) were calculated between 0 and 60 min; values in parentheses are exclusive of data from subject 4. See Glossary for definition of abbreviations.
 tolbutamide studies (studies I, II, and IV) correlated significantly with FSI ($r = 0.83$, $P < 0.001$ for regression analysis; Fig. 4; also confirmed using a nonparametric rank correlation test); the subjects with higher FSI tended to produce higher insulin levels in response to comparable tolbutamide concentrations.

Representative fits of the K/D model to the insulnergic effect of tolbutamide and calculated blood glucose profiles are shown in Fig. 5. Table 4 summarizes the population values for the K/D parameters of tolbutamide insulnergic effect and their variability. High values for Hill constants were obtained for the immediate insulnergic response mediated by serum tolbutamide and the delayed response mediated by the drug in the peripheral effect compartment.

Hypoglycemic effect of insulin in the presence and absence of tolbutamide. Representative fits of glucose disposal rate (with the assumptions of Eq. 2 in Appendix), together with corresponding calculated blood glucose profiles, are shown in Fig. 6. Statistical analysis indicated that addition of a hypoglycemic effect associated with serum insulin in the second model (Eq. 3), although reducing residuals, did not result in a significant improvement in the fit. Thus population estimates of parameters are reported only for the former model (Table 5).

An examination of individual parameter values indicated that insulin elimination from lymph in the presence of tolbutamide decreased significantly (7 cases) or was unchanged (6 cases) compared with the corresponding study with insulin. Also, during the studies with tolbutamide, GEI was increased in three cases and showed no change in six. No subject had a decreased GEI in the presence of tolbutamide. Similarly, ISI was increased (5 cases) or unchanged (8 cases) during the tolbutamide arms, and no individual showed a significant decrease.

Comparison of mean population values of $k_{0LYM}$, ISI, and GEI in the presence and absence of tolbutamide indicated that the drug enhances GEI ($P < 0.04$ and $P < 0.0001$ for study II vs. study III and study IV vs. study V, respectively). Also, when data from study IV were compared with those from study V, a significant ($P < 0.0001$) decrease in the elimination of insulin from lymph was estimated. The decreased insulin elimination during study II was of borderline significance ($P = \ldots$)

Table 4. Model parameters describing the insulnergic effect of tolbutamide

<table>
<thead>
<tr>
<th>Study</th>
<th>$S_{maxT}$</th>
<th>$C_{50T}$</th>
<th>$n_T$</th>
<th>$S_{maxE}$</th>
<th>$C_{50E}$</th>
<th>$n_E$</th>
<th>$k_{0E}$</th>
<th>$K_E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1,641*</td>
<td>170</td>
<td>24</td>
<td>1,499*</td>
<td>81</td>
<td>3</td>
<td>0.045</td>
<td>1.015</td>
</tr>
<tr>
<td>II</td>
<td>2,559*</td>
<td>173</td>
<td>60</td>
<td>2,519*</td>
<td>10</td>
<td>3</td>
<td>0.068</td>
<td>0.973</td>
</tr>
<tr>
<td>IV</td>
<td>1,074</td>
<td>192</td>
<td>36</td>
<td>365</td>
<td>64</td>
<td>3</td>
<td>0.040</td>
<td>1.022</td>
</tr>
</tbody>
</table>

*Values calculated after logarithmic transformation, because distribution of data was highly skewed.

See Glossary for definition of abbreviations.
Fig. 6. Model fits to hypoglycemic effect data (glucose consumption-production balance) and blood glucose levels in 2 representative subjects receiving tolbutamide (subjects 2 and 3, study II).

Despite a trend toward higher individual ISI values during the studies with tolbutamide (studies II and IV) than during the respective insulin studies (studies III and IV; Table 5), mean population values of the ISI differed only between studies IV and V (P < 0.0001). Values were not significantly different (P = 0.19) for study II vs. study III. The model parameters of glucose disposal expressed as their FSIGT equivalents are shown in Table 6.

Other Observations

Tolbutamide kinetics. Intersubject variability in serum drug concentrations was less than twofold and very similar in studies I, II, and IV (Fig. 3A). Thus kinetic parameters showed little inter- and intrasubject variability in comparison with the high variability in the insulinergic and hypoglycemic effects of the drug (Table 7).

C-peptide kinetics. When tolbutamide was used as the hypoglycemic agent, serum concentrations of C-peptide reached a maximum before 20 min (Fig. 3B), and the pattern of change in its secretion, described by deconvoluted spline functions, was similar (Fig. 7), indicating a common insulinergic mechanism(s). After an initial peak of serum C-peptide secretion, there was a second rise 40–60 min after the start of tolbutamide infusion. Thus a constant serum concentration of tolbutamide (Fig. 3A) was not associated with a stable C-peptide secretion (Fig. 7).

Serum C-peptide concentrations declined monotonically during administration of exogenous insulin (studies III and V; not shown). Monoeponential functions fitted to these concentrations (r = 0.840–0.998, median 0.954) indicated decay half-lives of 48 ± 5 and 27 ± 4 (SD) min in studies III and V, respectively (Table 8). The variability in decay rate was small within each study [coefficient of variation (CV) = 10 and 14% for studies III and IV, respectively], whereas AUCl values of C-peptide in response to tolbutamide were widely variable (Table 8). AUC at 19 min has been used by other investigators to assess β-cell function using insulin instead of C-peptide (18). Subject 4 had much higher serum concentrations of C-peptide in response to tolbutamide than the other individuals (mean ± 3.3 SD). High intersubject variability was observed in the secretogenic effect of tolbutamide (CV = 99%, n = 15), whereas intrasubject variation (CV) in this effect was 15% (n = 8).

Insulin Kinetics and Dynamics

As seen with C-peptide secretion, the serum insulin (Fig. 3C) and AUC values of serum insulin during tolbutamide administration (Table 8) indicated high inter- and low intrasubject variability. However, during insulin administration, intersubject variability in serum insulin and AUC values was much lower (Table 3). ANOVA showed no significant differences between calculated clearances during the euglycemic or hypoglycemic parts of the study. Clearance was similar in studies III and V. Variability in the estimated hepatic extraction.

Table 6. Intravenous glucose tolerance test equivalents of population values for model parameters

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Tolbutamide</th>
<th>Sgl × 10⁻³</th>
<th>Sg × 10⁻⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>No</td>
<td>8.8</td>
<td>7.1</td>
</tr>
<tr>
<td>I</td>
<td>Yes</td>
<td>10.2</td>
<td>10.0</td>
</tr>
<tr>
<td>V</td>
<td>Yes</td>
<td>10.0</td>
<td>8.6</td>
</tr>
<tr>
<td>IV</td>
<td>No</td>
<td>10.2</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>79.2</td>
<td>54.4</td>
</tr>
</tbody>
</table>

See Glossary for definition of abbreviations.
DISCUSSION

Sulfonylureas were first used to treat diabetes mellitus over one-half century ago. However, despite extensive investigation, their exact site and mode of action remain unclear (22). Although the acute insulinergic effect of sulfonylureas is beyond doubt (22), contradictory results have been reported with regard to their extrapancreatic and long-term insulinergic effects (22). Despite these uncertainties, tolbutamide is used as part of the implementation of the minimal model in FSIGT to improve parameter estimation by generating an extra peak of endogenous insulin (2, 38). This assumes that the drug itself does not change insulin sensitivity or glucose efficiency. However, when the results of the tolbutamide- and insulin-injection FSIGT are compared with results of clamp studies, it is clear that measures of ISI obtained by the two FSIGT methods, despite showing a good correlation with clamp-derived ISI values, are not concordant (7). Moreover, the values obtained from the two FSIGT methods are different from each other (31, 32). In the present study we have attempted to separate insulinergic and potential extrapancreatic effects of tolbutamide by combining population K/D modeling with an adapted minimal model of insulin and glucose dynamics.

First, we show that variability in the population mean values of kinetic parameters of tolbutamide is much less than the variability in its hypoglycemic effect and therefore cannot explain the wide variation in response. C-peptide concentration decreased during the administration of exogenous insulin (studies III and V), as expected if exogenous insulin suppresses production of endogenous insulin. The rate of decline was invariant within each study. Thus large differences in C-peptide level during tolbutamide arms (as indicated by AUCp) were attributed to a difference in C-peptide secretion rather than in its disposition. Observation of similar individual C-peptide disposition is consistent with claims that the use of population mean values of kinetic parameters describing the elimination of C-peptide for the purpose of deconvolution produces results similar to those obtained when individual parameter values of C-peptide elimination are used (34). Indeed, previous estimates of the kinetic parameters of C-peptide are highly consistent (9, 29, 34).

The result of multiple regression analysis of glucose disposal rate during the studies with exogenous insulin was consistent with knowledge of factors influencing insulin sensitivity (e.g., weight, sex, and age). This analysis also showed that subjects with a higher muscle-to-fat ratio should have a greater hypoglycemic response.

A stable concentration of tolbutamide was not associated with stable secretion of insulin. This is consistent with the findings of Lewis et al. (20), who showed that only an increasing concentration of tolbutamide, obtained by stepwise administration of multiple doses, could produce a stable insulin secretion. Despite similar serum tolbutamide concentrations in different individuals, the insulinergic response was highly variable between subjects, one of whom (subject 4) clearly showed a greater effect than the others. Surprisingly, the hypoglycemic effect of the drug in this subject was no greater than that in the others and was reproducibly in the middle of the range for dextrose requirement (46–60th percentile). Further inspection of the data indicated that the insulinergic response to tolbutamide was related to the fasting level of insulin. The latter increases in response to impaired insulin sensitivity (19, 23), such that blood glucose is maintained in the normal range. This could explain why individuals such as subject 4, despite having higher insulin secretion in response to tolbutamide, do not produce a greater hypoglycemic effect. The insulin resistance of subject 4 was confirmed during the clamp study with insulin, when, despite having

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**Table 7. Population kinetic parameters of tolbutamide**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Avg</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (ml/min)</td>
<td>27.1</td>
<td>6.9</td>
</tr>
<tr>
<td>V (liters)</td>
<td>6.9</td>
<td>7.1</td>
</tr>
<tr>
<td>k11, min⁻¹×10⁻²</td>
<td>8.7</td>
<td>8.4</td>
</tr>
<tr>
<td>k12, min⁻¹×10⁻²</td>
<td>73</td>
<td>34</td>
</tr>
<tr>
<td>f₀, %</td>
<td>56</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 7. Population kinetic parameters of tolbutamide.

See Glossary for definition of abbreviations.
Table 8. Model independent parameters describing C-peptide and insulin kinetics

<table>
<thead>
<tr>
<th></th>
<th>C-Peptide AUC&lt;sub&gt;0-20&lt;/sub&gt;, mmol·l&lt;sup&gt;-1·min&lt;/sup&gt;</th>
<th>C-Peptide Half-Life, min</th>
<th>Steady-State Serum Insulin, mU/l</th>
<th>Insulin Clearance, l/min</th>
<th>Insulin Hepatic Extraction Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study I</td>
<td>Study II</td>
<td>Study IV</td>
<td>Study III</td>
<td>Study V</td>
</tr>
<tr>
<td>Average</td>
<td>48.8</td>
<td>53.3</td>
<td>31.5</td>
<td>48.4</td>
<td>26.6</td>
</tr>
<tr>
<td>CV, %</td>
<td>87</td>
<td>73</td>
<td>24</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>

AUC<sub>0-20</sub> area under curve from 0 to 20 min.

representative serum insulin levels, the subject required the lowest infusion rate of dextrose to maintain the target blood glucose concentration. A compensatory high insulinergetic response to glucose in insulin-resistant subjects is indicated when the product of insulin secretion and ISI as measured by the minimal model remains fixed (17). Therefore, a hyperbolic relationship exists between β-cell function and ISI as measured by the minimal model (17). This confirms the in vitro observation that the drug mimics the insulinergetic effect of glucose action in releasing insulin from the pancreas (10). Thus compensatory mechanisms of insulin resistance are common to glucose and tolbutamide.

Two other important observations offered by the model with regard to insulin secretion were the "all-or-none" responses indicated by large Hill coefficients and an explanation for biphasic secretion of insulin as well as the suppressive effect of hypoglycemia. These observations confirm the findings of animal studies (13), explain the difficulty encountered in establishing a dose-response curve for the insulinergetic effects of tolbutamide (22), and suggest a homeostatic defense mechanism against severe hypoglycemia caused by tolbutamide. The latter may account for the low incidence of hypoglycemia in the clinical use of tolbutamide compared with other sulfonylureas (16).

Perhaps the most important findings of this study were related to extrapancreatic effects of tolbutamide. In a model-independent analysis, we showed that the effect of insulin may be prolonged by tolbutamide, despite the fact that the elimination of insulin from serum is unaffected (30). The present study confirms this by showing a significant decrease in the elimination rate constant of insulin from a remote effect compartment, which explains the prolongation of effects of insulin. The half-life of insulin elimination from this compartment increased from 14 to 21 min (study III vs. study II) and from 3 to 19 min (study V vs. study IV). Our analysis reaffirms that serum insulin plays an insignificant role in the overall economy of glucose, since a model in which hypoglycemic effect was assumed to reflect serum and peripheral (lymph) concentrations of insulin was no better than the conventional representation of the effect mediated in a peripheral compartment only. This observation also serves to validate the reliability of our new model.

A significant increase in GEI was observed during the clamps with tolbutamide. However, ISI values varied significantly in the presence of the drug in only one of the study groups. Both effects were widely variable (Table 5). Conversion of our GEI and ISI values to FSIGT equivalents, assuming a mean population value for glucose volume of distribution (for details see APPENDIX), resulted in values within the range previously obtained by application of the minimal model to data from healthy subjects (4–7, 17, 32, 33). The subjects of studies I–III responded modestly to the proposed extrapancreatic effect of tolbutamide relative to those who took part in studies IV and V (only 3 subjects completed all studies). A notable difference between these two groups was the higher insulin sensitivity (as indicated by the 1RI; Table 2) of the second group, many of whom were accustomed to regular exercise. It is also possible that the different response may have been related to the time course of the effects, inasmuch as studies IV and V were shorter than studies I–III (Table 1). This may suggest that these effects of tolbutamide are of short duration and cannot be measured easily over long periods of time.

In vivo evidence for a direct effect of tolbutamide remains controversial. In a recent in vivo study by Lewis et al. (20), the serum concentration of glucose during infusion of tolbutamide showed a decline in patients with insulin-dependent diabetes mellitus (Fig. 4 in Ref. 20), which could imply a direct effect of tolbutamide. However, the authors did not perform a trend analysis of their data. Although the effects of tolbutamide on GEI and ISI as measured by the minimal model (SI) shown in our study may be attributed to direct effects of tolbutamide, they may equally reflect large differences between peripheral and portal insulin concentrations. Also, the presence of additional proinsulin and C-peptide during tolbutamide administration may play a part. Application of the minimal model to FSIGT with tolbutamide injection results in SI values that are generally similar to those obtained by clamp studies: only slightly higher in some cases (6) and slightly lower in others (31). However, the original (14, 35) and more recent FSIGT studies using the insulin-injection protocol (31, 32) reported SI values lower than those found in clamp studies. Thus values from FSIGT with insulin injection are correlated with those from clamp studies but are not concordant (31, 32). The possibility of a tolbutamide effect on insulin sensitivity (but not glucose efficiency) has been suggested earlier (4), but it was assumed that any systematic change would pose no problem in comparative studies. The need for further studies to assess the
importance of the difference between tolbutamide- and insulin-injection FSIGT protocols has been emphasized (4, 32). Almost equivalent Si values from FSIGT with tolbutamide injection and clamp studies but lower Si values from FSIGT with insulin injection (31, 32) suggest that tolbutamide injection may raise the true Si values. Studies in which the same individuals received tolbutamide- and insulin-injection FSIGT in a crossover design had not been reported before our analysis. However, Saad et al. (32) recently published the results of such a study where Si and GEI as measured by the minimal model (Sg) from tolbutamide-modified FSIGT were compared with results from an insulin-modified FSIGT in the same group of subjects. The findings of Saad et al. were essentially in agreement with the results of our analysis, in that they observed substantial differences between the two protocols. Although the Si value from the tolbutamide protocol gave a quantitative measure of insulin action nearly equivalent (13% lower) to that from the glucose clamp (the gold standard), the estimates from the insulin protocol were 44% lower than those from the glucose clamp. They also noted that the time course of insulin action was more prolonged in the presence of tolbutamide, an effect that was explained by changes in $k_{0Lym}$ in our study.

With respect to glucose effectiveness, and in contrast to our observation, Saad et al. (32) found no significant difference between GEI in the presence or absence of tolbutamide. However, they suggested that this could be due to the fact that Sg is estimated mainly from early glucose data, when no tolbutamide is present. Thus lack of a difference in GEI determined from two protocols does not exclude the possibility of a tolbutamide effect on GEI.

Conclusion

Application of a population approach to mathematical modeling in endocrinology proved to be successful, inasmuch as many of the findings with respect to insulin secretion, C-peptide kinetics, covariates for hypoglycemic effects of insulin, and the suppressive effect of hypoglycemia on insulinergic effects of tolbutamide were consistent with previous reports based on classical data analysis. Moreover, this approach afforded two new findings.

1) Variability in the insulinergic effect of tolbutamide is related to the insulin sensitivity of subjects. This is similar to the compensatory high insulinergic response to glucose in insulin-resistant subjects and suggests that compensatory mechanisms of insulin resistance are common to glucose and tolbutamide insulinergic effects.

2) Tolbutamide has extrapancreatic effects, inasmuch as it prolongs the effect of insulin in a remote effect compartment (lymph) and may change ISI and GEI of glucose economy.

These effects may arise purely from direct effects of tolbutamide, or they may reflect the portal-to-peripheral ratio of serum insulin.

The results of this study should encourage a wider use of the population approach in mathematical modeling in endocrinology. They also call for a reevaluation of the in vivo effects of tolbutamide and reaffirm the view that measures of insulin sensitivity and glucose efficiency from tolbutamide and insulin injection protocols are not directly comparable.

APPENDIX

Main Model Fits

The two main model fits were 1) insulin secretion (in response to tolbutamide infusion) vs. time

\[
\text{insulin secretion} = f_1[\text{Sec}_0, f_2(C_{TB}, \text{Sec}_{S_{max}}, C_{S_{50}}, n_S), f_2 (C_{E_{TB}}, \text{Sec}_{E_{max}}, C_{E_{50}}, n_E), BG, BG_0, Kg]
\]

and 2) glucose disposal vs. time

\[
d(C/P)/dt = f_3(\Delta C_{InsLym, Lym}, BG, BG_0, Sg)
\]

\[
d(C/P)/dt = (\Delta C_{InsLym} \times BG \times Si_{Lym}) + (BG - BG_0) \times Sg
\] or

\[
d(C/P)/dt = f_3(\Delta C_{InsLym, Lym}, \Delta C_{InsS}, Si_S, BG, BG_0, Sg)
\]

\[
d(C/P)/dt = (\Delta C_{InsS} \times BG \times Si_S) + (BG - BG_0) \times Sg
\]
Functions Describing Main Model Parameters

Some of the parameters of the above models were not
calculated with time. These included the concentrations of
tolbutamide and insulin in serum, blood glucose level, and the
concentrations of tolbutamide and insulin in remote compart-
ments (effect compartment and lymph compartment, respec-
tively). The time profiles of the first three of these parameters
were fitted by appropriate equations, and the individual
values of variables for each fit were subsequently entered as
covariates for individuals into the second stage (main fit).

Variables determining the two latter profiles were calculated
during the main fit (K/D link modeling). Thus simulation of
the time profiles for these concentrations was bypassed, since
the variables were obtained from the time profiles of dynamic
effect.

Equations describing each of the parameters used within
the main fits are as follows.

Serum tobutamide concentration vs. time (fitted in the 1st
stage).

\[
C_{TB} = \frac{k_0 \text{ dose}}{V_c} + \sum_{i=1}^{2} \left( \frac{k_0 \text{ dose}}{V_c} \times \frac{1}{\lambda_1} \sum_{i=1}^{2} \left( \frac{1 - e^{\lambda_i t}}{\lambda_i - \lambda_1} \right) \right)
\]

Tolbutamide concentration in a remote effect compartment
(incorporated into the K/ D link model and fitted during the
2nd stage).

\[
C_{EB} = \frac{k_0 \text{ dose}}{V_c} \times \sum_{i=1}^{2} \left( \frac{1 - e^{\lambda_i t}}{\lambda_i - \lambda_1} \right)
\]

Blood glucose level (fitted in the 1st stage). For studies II – V
(euglycemia and subsequently hypoglycemia)

\[
BG = f_6(BG_0, A_1, A_2, B_1, B_2, T_{hypor}, t)
\]

when \( t < T_{hypor} \)

\[
BG = BG_0 + A_1(e^{-B_1t} - e^{-B_2t})
\]

when \( t > T_{hypor} \)

\[
BG = BG_0 + A_1(e^{-B_1t} - e^{-B_2t}) + A_2(e^{-B_{hypor}t} - e^{-B_{hypor}T_{hypor}})
\]

and for study I (euglycemia)

\[
BG = f_6(BG_0, A_1, B_1, B_2, T_{lagr}, t)
\]

\[
BG = BG_0 + A_1 \cos (B_1(t - T_{lagr})) \cos (B_2(t - T_{lagr}))
\]

Serum insulin concentration (fitted in the 1st stage). For
studies I, II, and IV (tolbutamide arms of studies)

\[
C_{ins} = f_{10}(C_{ins0}, A_1, A_2, B_1, B_2, T_{lagr}, t)
\]

when \( t < T_{lagr} \)

\[
C_{ins} = C_{ins0} + A_1(e^{-B_1t} - e^{-B_2t})
\]

when \( t > T_{lagr} \)

\[
C_{ins} = C_{ins0} + A_1(e^{-B_1t} - e^{-B_2t}) + A_2(e^{-B_{lagr}t} - e^{-B_{lagr}T_{lagr}})
\]

and for studies III and V (insulin arms of studies)

\[
C_{ins} = f_{12}(C_{ins0}, C_{load}, k_{e_1}, T_{load}, t)
\]

when \( t <= T_{load} \)

\[
C_{ins} = C_{ins0} e^{-k_{e_1} t} + C_{ins1}(1 - e^{-k_{e_1} t})
\]

when \( t >= T_{load} \)

\[
C_{ins} = C_{ins0} e^{-k_{e_1} T_{load}} + C_{ins1}(1 - e^{-k_{e_1} t}) e^{-k_{e_1} (t - T_{load})} + C_{ins2}(1 - e^{-k_{e_1} (t - T_{load})})
\]

Lymph insulin concentration (incorporated into the K/ D
link model). The concentration of insulin in lymph was a function
of its concentration in serum, and the transfer rates between the
central and remote (lymph) compartment for insulin

\[
C_{ins Lym} = f_{13}(C_{ins S}, F_{Lym}, k_{0Lym})
\]

With the assumption of first-order transfer rates of insulin to
and from the peripheral compartment \((k_{Lym1} \text{ and } k_{0Lym})\), the
rate of change of insulin concentration in lymph (peripheral
compartment) was described by

\[
\frac{dC_{ins Lym}}{dt} = k_{Lym1} C_{ins} + k_{0Lym} C_{ins Lym}
\]

It was also assumed that exit of insulin from the peripheral
arm(s) of the studies was then calculated as follows

\[
\frac{d(C/P)}{dt}_{\text{midtime}} = \frac{(C/P)_{t_1} - (C/P)_{t_2}}{\Delta t} \tag{19}
\]

where the subscript \text{ss} indicates steady-state condition (or state of equilibrium between mass in 2 compartments). \(F_{\text{Lym}}\) has been reported to be 0.6–0.7 (1, 37). We used a fixed value of 0.67.

The concentration of insulin in lymph could then be calculated as follows

\[
F_{\text{Lym}} \cdot \frac{dC_{\text{Ins Lym}}}{dt} = k_{\text{Lym}} \cdot C_{\text{Ins ss Lym}} - k_{0,\text{Lym}} \cdot C_{\text{Ins Lym}}
\]

Hepatic extraction and clearance of insulin. Individual values of insulin clearance were calculated from the insulin concentration in lymph and output from the peripheral compartment should be equal, Eq. 13 was simplified to contain only one rate constant

\[
\left\{ \begin{align*}
k_{\text{Lym}} \cdot C_{\text{Ins ss Lym}} &= k_{0,\text{Lym}} \cdot C_{\text{Ins ss Lym}} \\
C_{\text{Ins ss Lym}} &= C_{\text{Ins ss}} \cdot F_{\text{Lym}} \\
\frac{dC_{\text{Ins Lym}}}{dt} &= k_{\text{Lym}} \cdot C_{\text{Ins ss Lym}} - k_{0,\text{Lym}} \cdot C_{\text{Ins Lym}}
\end{align*} \right.
\]

Converting GEI and ISI to their classical minimal model equivalents. The values of GEI and ISI derived from our model are readily converted to corresponding values of \(S_{\text{g}}\) and \(S_{\text{i}}\) obtained in FSIGT experiments by multiplying our values by the central volume of distribution of glucose. For example, with the assumption of a value for the latter of 1.58 dl/kg (11), the average \(S_{\text{g}}(\text{FSIGT})\) in study III (absence of tolbutamide) is calculated as follows

\[
\text{GEI} = 1.00 \text{ (dl/min)} \quad S_{\text{g}}(\text{FSIGT}) = \frac{\text{GEI}}{V_{\text{BG}}} = 1.00/(1.58 \times 74) = 8.58 \times 10^{-3} \text{ (min}^{-1})
\]

\[
\text{ISI} = 0.12 \text{ (min}^{-1} \cdot \text{dl} \cdot \text{mU}^{-1} \cdot \text{I}^{-1}) \quad S_{\text{i}}(\text{FSIGT}) = \frac{F_{\text{Lym}} \cdot \text{GEI}}{V_{\text{BG}}} = \frac{(0.67 \times 0.12)}{(1.58 \times 74)} = 6.9 \times 10^{-4} \text{ (min}^{-1} \cdot \text{mU}^{-1} \cdot \text{I}^{-1})
\]

Population Model Structure

In the population analysis the \(j\)th measurement (e.g., \(C_{\text{TB}}\)) for the \(i\)th individual (\(y_{ij}\)) is related to the model parameters by the following expression

\[
y_{ij} = f(\phi_i) + \epsilon_{ij} \tag{20}
\]

where \(f\) is a function (Eq. 4) describing the expected value of the response for a given parameter vector \(\phi_i\) (e.g., \(k_{0,\text{dose}}, V_{\text{c}}, \lambda_1, \lambda_2, \lambda_3, T\)). The term \(\epsilon_{ij}\) accounts for the (random) error between the true value and the corresponding measurement and is modeled as follows

\[
\epsilon_{ij} \sim N(0, \sigma^2 \times y_{ij}^\beta) \tag{21}
\]

\[
\phi_i = g(\theta, \chi_i) + \eta_i \tag{22}
\]

where \(g\) is a known function describing the expected value of \(\phi_i\) as a function of individual covariates \(\chi_i\) and the vector of
true population parameters $\theta$ and $v_i$ determines the interindividual variability of the parameter and is assumed to have a normal distribution with a mean of zero and a variance of $\gamma \sigma^2 \theta^T$

$$\eta_i = N(0, \gamma^2 \theta^T)$$

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