Glycogenesis and glucose oxidation during an intravenous glucose tolerance test in man

Leslie J. C. BLUCK, Allan T. CLAPPERTON, Cheryl V. KIDNEY and W. Andy COWARD
MRC Human Nutrition Research, Elsie Widdowson Laboratory, Peterhouse Park, Fulbourn Road, Cambridge CB1 9NL, U.K.

ABSTRACT

The quantity of deuterated glucose customarily given in labelled IVGTTs (intravenous glucose tolerance tests) changes the isotopic composition of the subject's body water enough to be detected by mass spectrometric techniques. Glucose undergoing direct glycogenesis does not contribute label to the body water pool, and isotope incorporated into it must have come from glucose that has either been oxidized or undergone indirect glycogenesis. By subtracting the amount of label found in body water from the total amount of glucose utilized, as calculated from the minimal model of glucose disappearance, it should be possible to study the partitioning of the dose given between direct glycogenesis in skeletal muscle and other metabolic pathways. To establish these principles, we used isotope ratio MS to determine body water composition in groups of healthy (n = 7; mean weight, 76 kg; fasting plasma glucose and insulin, 5.1 mmol and 40 pmol respectively) and Type II diabetic (n = 5; mean weight, 84 kg; fasting plasma glucose and insulin, 6.2 mmol and 75 pmol respectively) subjects undergoing IVGTTs. It was found that, for healthy subjects, 31% of the dose given was utilized in direct glycogenesis and this was decreased to 15% in diabetes. Defects in muscle glycogen synthesis in diabetes of the same order are well known from magnetic resonance studies. We conclude that measurement of label incorporation into body water is potentially useful for investigation of the metabolism of a glucose load in vivo during an IVGTT.

INTRODUCTION

The quantity of deuterated glucose (approx. 2 g, with 17.5 g of unlabelled material) customarily given in labelled IVGTTs (intravenous glucose tolerance tests) will theoretically change the isotopic composition of a subject's body water sufficiently to be detected by sensitive IRMS (isotope ratio mass spectrometry) techniques. If the [2H] label is incorporated in this way, it must have come from a substrate that has either been oxidized or undergone indirect glycogenolysis and, thus, the kinetics of its appearance could be used to study the metabolic processes involved. The revised two-compartment minimal model of glucose kinetics [1] could, therefore, be used to conventionally calculate both insulin-assisted and insulin-independent clearance, but, at the same time, IRMS measurement of the appearance of deuterium in the body water may give information on the fraction of glucose not participating in direct glycogenesis. Under conditions of hyperglycaemia/hyperinsulinaemia, the combination of oxidation and skeletal muscle glycogen accounts for all of the glucose utilization in both healthy and diabetic subjects [2]; thus, the difference between total glucose disposal (the minimal model data) and glucose oxidation ([2H]-incorporation data) could provide insights into the simultaneous disposal of glucose via the direct glycogenic pathway.

The present studies were intended to establish these principles in experiments in which SG (glucose effectiveness) and SI (insulin sensitivity) were being...
measured in subjects with normal and impaired glucose tolerance.

MATERIALS AND METHODS

Subjects
The study protocol was approved by the Cambridge Local Research Ethics Committee. Potential recruits, non-smoking males aged 30–65 years, were sent an information sheet describing the purpose of the study and volunteer commitment and a brief questionnaire asking about height, weight and current medication. Current use of drugs expected to interfere with glucose metabolism was taken as an exclusion criterion; users of aspirin in moderation (300 mg/day or less), antihistamines and calcium channel blocking drugs were not excluded. Informed written consent was obtained from the volunteers, who were then invited to MRC Human Nutrition Research (HNR) to have an OGTT (oral glucose tolerance test) to determine their glucose tolerance status, unless they had been diagnosed previously as diabetic and were being currently treated by a regime of diet and exercise only. Two subgroups were then selected, one comprising seven lean (BMI < 25 kg·m⁻², where BMI is body mass index) individuals with normal glucose tolerance, and five subjects with diabetes, as defined by WHO (World Health Organization) criteria.

Intravenous glucose doses
Glucose doses were prepared by Stockport Pharmaceuticals (Stockport, U.K.) by adding 1.75 g of 6,6-[²H₂]glucose and 0.120 g of 1-[¹³C]glucose (both certified pyrogen-free and obtained from Promochem Ltd, Welwyn Garden City, Herts., U.K.) to 19 g of unlabelled glucose in the form of a 50 % (w/v) aqueous solution. The carbon-labelled material was used for use in a contemporaneous comparative study of insulin-resistance data from [³H]- and [¹³C]-labelled glucose. Insulin doses were prepared from human Actrapid insulin and diluting medium for soluble insulin injection (both from Novo Nordisk, Crawley, West, Sussex, U.K.) to give a 2.8 μmol solution, which was divided into 0.5 ml aliquots for intravenous administration of 1.4 nmol (175 m-units) insulin.

IVGTT protocol
Subjects were invited to participate in two IVGTTs, separated by at least 4 weeks, in order to determine the repeatability of the model estimates for the kinetic parameters. On each occasion, they were asked to attend the volunteer suite at HNR on the morning of the day of the test, having abstained from food and drink (other than water) for 12 h. Before participating in any clinical procedures, subjects were asked to provide a urine sample to test for glycosuria. A frequently sampled insulin-modified IVGTT was then performed [3].

Sample analysis
The isotopic composition of the plasma glucose was measured by forming the α-D-glucofuranose cyclic 1,2,3,5-bis(butylboronate)-6-acetate derivative for chromatographic separation [4], and using GC/MS for determination of the deuterated species. Full details of the analytical procedures, including plasma glucose and insulin measurements, have been given elsewhere [3].

Plasma water was analysed for deuterium by equilibration with hydrogen gas using a platinum catalyst [5].

Models and calculations

Two-compartment minimal model
Although the labelled IVGTT is frequently interpreted on the basis of the original (one-compartment) minimal model [6, 7], non-physiological results are usually obtained for endogenous glucose production. A more sophisticated two-compartment minimal model has been proposed to overcome this problem [8]. This model in its original guise provides realistic estimates of both S₀ and S₁ [9], but it sometimes produces physiologically impossible estimates of glucose disposal. Recently, this issue has been addressed [1] by slight adjustment to the internal constraints within the model. In the present study, elements to allow for urinary glucose losses were also added. The model and the fitting procedures used are completely described in the Appendix.

[^2H] recovery
Incremental isotope ratio was converted into absolute quantity of deuterium by multiplication by the isotope distribution space and fraction of dose recovered calculated. Distribution space was estimated from BMI [10]

\[
\text{Fraction lean tissue} = 1 - \text{fraction fat} \\
= 1.478 - 0.0376 \cdot \text{BMI} + 0.004 \cdot \text{BMI}^2
\]

[^2H] distribution space = 1.04 \times 0.73 \times \text{weight} \\
\times \text{fractional lean tissue}

These equations assume that lean tissue is 73 % water and a factor of 1.04 is applied to allow for the known offset of [²H] distribution space compared with total body water.

The time courses for the fraction of dose in plasma were fitted to a quadratic spline, which is well suited for the description of sigmoidal curves. This provides two easily interpretable parameters, \( t_1 \) (the time at which the first node in the spline occurs), corresponding to the maximum isotope appearance rate, and \( t_2 \) (the time of the second node), which corresponds to when no more label is entering the plasma, from which a \( t_{1/2} \) (half time) for the process can be calculated [11]. The fitted plateau enrichment was used to estimate the quantity of glucose directly oxidized or participating in indirect glycogen formation. This, by subtraction from the estimate of total glucose disposal from the minimal model, gives
Table 1  Anthropometric measurements, fasting plasma glucose and insulin concentrations, $S_G$ and glucose sensitivity for the two groups

All values are means $\pm$ S.E.M., except for $^*$, where values are means $\pm$ S.D. $^{†}P < 0.001$ compared with normal subjects.

<table>
<thead>
<tr>
<th></th>
<th>Normal group</th>
<th>Diabetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76 $\pm$ 6</td>
<td>84 $\pm$ 13</td>
</tr>
<tr>
<td>BMI (kg m$^{-2}$)</td>
<td>24.1 $\pm$ 0.5</td>
<td>25.6 $\pm$ 2.6</td>
</tr>
<tr>
<td>Fasted plasma glucose (mmol)</td>
<td>5.1 $\pm$ 0.1</td>
<td>6.2 $\pm$ 0.3 $^{†}$</td>
</tr>
<tr>
<td>Fasted plasma insulin (pmol)</td>
<td>40 $\pm$ 6</td>
<td>75 $\pm$ 19</td>
</tr>
<tr>
<td>$S_G$ (h$^{-1}$)</td>
<td>0.510 $\pm$ 0.046</td>
<td>0.0095 $\pm$ 0.0012 $^{†}$</td>
</tr>
<tr>
<td>$S_I\cdot V_I$ (pmol h$^{-1}$)</td>
<td>0.197 $\pm$ 0.016</td>
<td>0.0023 $\pm$ 0.0005 $^{†}$</td>
</tr>
</tbody>
</table>

Table 2  Partitioning of the glucose given in the IVGTT by various pathways

Values are means $\pm$ S.E.M.

<table>
<thead>
<tr>
<th>Pathways of partitioning</th>
<th>Normal group</th>
<th>Diabetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lost in urine</td>
<td>1.5 $\pm$ 0.2</td>
<td>3.7 $\pm$ 0.7</td>
</tr>
<tr>
<td>Utilized from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (zeroth order)</td>
<td>24.4 $\pm$ 1.2</td>
<td>46.9 $\pm$ 1.6</td>
</tr>
<tr>
<td>Plasma (first order)</td>
<td>18.6 $\pm$ 0.8</td>
<td>7.3 $\pm$ 0.4</td>
</tr>
<tr>
<td>Remote pool</td>
<td>46.3 $\pm$ 3.0</td>
<td>27.0 $\pm$ 2.6</td>
</tr>
<tr>
<td>Used in glycogenesis</td>
<td>30.8 $\pm$ 2.9</td>
<td>14.9 $\pm$ 3.1</td>
</tr>
</tbody>
</table>

Table 3  Total quantities of glucose metabolized by various pathways during the whole 5 h of the IVGTT

Values are means $\pm$ S.E.M.

<table>
<thead>
<tr>
<th>Pathways of metabolism</th>
<th>Normal group</th>
<th>Diabetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lost in urine</td>
<td>23 $\pm$ 3</td>
<td>53 $\pm$ 11</td>
</tr>
<tr>
<td>Utilized from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (zeroth order)</td>
<td>372 $\pm$ 19</td>
<td>657 $\pm$ 43</td>
</tr>
<tr>
<td>Plasma (first order)</td>
<td>283 $\pm$ 12</td>
<td>103 $\pm$ 7</td>
</tr>
<tr>
<td>Remote pool</td>
<td>709 $\pm$ 48</td>
<td>360 $\pm$ 32</td>
</tr>
<tr>
<td>Used in glycogenesis</td>
<td>468 $\pm$ 40</td>
<td>202 $\pm$ 38</td>
</tr>
<tr>
<td>Total utilized from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (zeroth order)</td>
<td>1290 $\pm$ 81</td>
<td>1846 $\pm$ 106</td>
</tr>
<tr>
<td>Plasma (first order)</td>
<td>835 $\pm$ 48</td>
<td>254 $\pm$ 16</td>
</tr>
</tbody>
</table>

Figure 1  Typical appearance curve of the deuterium label from the dose in plasma as determined by IRMS techniques

In all cases, the quantity of deuterium-labelled substrate given in the standard labelled IVGTT protocol was sufficient for it to be observed entering the body water pool using standard IRMS techniques; however, to our knowledge, this is the first report of the measurement of body water enrichment occurring as a consequence of such a test.

A typical time course for the appearance of label in plasma is given in Figure 1. The average fraction of the dose recovered in the body water was 58 $\pm$ 2% for the healthy subjects, significantly different from the 66 $\pm$ 3% observed for the diabetic subjects; however, normalizing by subject weight removes the significance of the comparison: the average values for healthy and diabetic subjects being 897 $\pm$ 40 and 926 $\pm$ 57 $\mu$mol/kg of body weight respectively.

The maximal rate of appearance of label in plasma occurred at approx. 20 min post dose, and the concentration of deuterium reached a plateau after 4 or 5 h. No significant differences were found for any of the parameters $t_1$, $t_2$ or $t_{1/2}$ between the two groups, nor did any of them correlate significantly with $S_I$. However,
Table 4  Intra- and inter-subject variabilities

<table>
<thead>
<tr>
<th></th>
<th>Glucose dose (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normal group</td>
</tr>
<tr>
<td></td>
<td>Intra-subject variability</td>
</tr>
<tr>
<td>Recovered in body water</td>
<td>4.6</td>
</tr>
<tr>
<td>Lost in urine</td>
<td>0.4</td>
</tr>
<tr>
<td>Utilized from</td>
<td></td>
</tr>
<tr>
<td>Plasma (zeroth order)</td>
<td>3.2</td>
</tr>
<tr>
<td>Plasma (first order)</td>
<td>2.5</td>
</tr>
<tr>
<td>Remote pool</td>
<td>9.7</td>
</tr>
<tr>
<td>Total accounted for</td>
<td>4.9</td>
</tr>
<tr>
<td>Total utilized</td>
<td>4.7</td>
</tr>
<tr>
<td>Used in direct glycogen formation</td>
<td>7.7</td>
</tr>
<tr>
<td>$S_1$ (h$^{-1}$)</td>
<td>0.0069</td>
</tr>
<tr>
<td>$S_G$ (pmol · h$^{-1}$)</td>
<td>0.0016</td>
</tr>
</tbody>
</table>

there was a negative correlation ($r \approx -0.4$) between $t_{1/2}$ and $S_G$ ($P < 0.05$), indicating that it was the pathways independent of insulin action which provided the main contribution to the label appearing in body water.

The measures of repeatability obtained from the duplicate measurements are given in Table 4. The intra-subject reproducibility was similar to that reported previously for $S_1$ measurement by the two-compartment model (coefficient of variance for $S_1$ of approx. 20 % [12]).

**DISCUSSION**

We believe that the interpretation of IVGTT data by the two-compartment model in the present study is unique in that it is the first report performed in populations with known differences in their response to a glycaemic load which has analysed data using the two-compartment minimal model in its most recent version and with constraints chosen appropriate to the study groups. It has also been demonstrated that direct estimate of the fraction of the dose oxidized can be made with the use of data from $^{2}$H incorporation in body water, and then by inference the proportion of glucose dose stored by skeletal muscle as glycogen. Urinary losses were included in the model to permit complete calculation of glucose disposal. Inclusion of the estimates of glycogenolysis add considerably to the power of the IVGTT assessment of glycaemic response without increasing the complexity of the clinical protocol or imposing additional burden on the subjects.

**Indices of $S_1$ and $S_G$**

As expected, there is a clear difference in the $S_1$s between the two groups. However, in contrast with the recent study in a similar sized Japanese population [13], we also found that $S_G$ deduced from the two-compartment minimal model was significantly decreased in diabetes.

The discrepancy between the findings of these two studies may be explained by the version of the two-compartment model used. In its original form, which was used in the latter study [13], a value of $\beta = 3$ was adopted and the zeroth-order rate of utilization of plasma glucose was taken as a constant value for all study populations. If the present data is analysed using values of $\alpha$ and $\beta$ appropriate to normal subjects universally, then, although a slight decrease in $S_G$ (approx. 7 %) in diabetes is observed, it is too small to be considered significant with these study numbers.

Impairment of both insulin-independent and -dependent glucose metabolism is expected in the diseased state [14], due to a peripheral deficit of glucose uptake. It is well documented that, when measured by the unlabelled minimal model, such perturbations of $S_G$ are observed in the diseased states of impaired glucose tolerance (approx. 40 % decrease [15]), Type II diabetes (approx. 50 % decrease [16]) and Type I diabetes (approx. 40 % decrease [17]). Surprisingly, there is a paucity of studies comparing normal and diabetic subjects using tracer techniques. Avogaro and co-workers [18] used the one-compartment model to analyse labelled IVGTT data in diabetic subjects, obtaining a mean $\pm$ S.E.M. for $S_G$ of $0.318 \pm 0.029$ h$^{-1}$, decreased significantly by 27 % from that found for normal subjects ($0.439 \pm 0.036$ h$^{-1}$) by the same group [19]. In contrast with the unlabelled model, which is indicative of the sum of peripheral insulin-independent glucose uptake and suppression of endogenous glucose release, $S_G$ obtained from isotope experiments represents uptake only, which has been used as an explanation for why a smaller effect is obtained from the label data.

In the study by Nagasaka et al. [13], which to our knowledge is the only other two-compartment comparison made between diabetic and normal subjects, this argument was used to explain the apparent unimpaired
Glycogenesis and oxidation during an intravenous glucose tolerance test

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The measures of glycogenesis obtained during IVGTT do not correlate sufficiently well with $S_0$ to reach significance with a study group of this size; however, the correlation with $S_0$ (after the customary square root transformation) is significant ($P < 0.001$) which, at first
sight, is surprising. However, \( S_G \) is not solely a measure of insulin-independent glucose clearance, as is often believed, but, as has been pointed out in discussions of the one-compartment minimal model [28], has within it a large component reflecting insulin action at basal levels. This is immediately apparent in our formulation for the two-compartment model from eqn (7) in the Appendix, which indicates that, for any particular population group, \( S_G \) is proportional to the quotient of insulin-assisted glucose metabolism and the plasma glucose pool size in the basal state. In the fasted state, when the principal fuel used by muscle is fatty acids, defects in the insulin-assisted muscle glycogen pathway will have a profound effect on the flux of glucose lost from the remote compartment and, hence, on the observed value of \( S_G \).

**Significance of urinary glucose loss**

It is not customary to include losses due to glycosuria in the structure of minimal models, even though the renal threshold is usually exceeded for part of the test period. Avogaro et al. [18] investigated the effects of glucose urinary loss on the parameters obtained from the one-compartment model, but, to our knowledge, the present study is the first to report a similar modification to the two-compartment model.

We found that, for normal subjects, losses via this route amounted to only 1.5 ± 0.2 % of the dose given, whereas, urinary losses rose to 3.7 ± 0.7 % in the diabetic group. This is considerably less than the approx. 17 % losses we calculate from the data given for diabetic subjects in the study by Avogaro et al. [18]; however, in that study, the glucose dose given was approx. 20 % bigger than that used in the present study.

Explicit inclusion of urinary losses had little effect on the results from the minimal model for the healthy subjects, both \( S_G \) and \( S_I \) appear to be underestimated by its omission, but not to significant levels as judged by paired Student \( t \) tests. Likewise, the relative portions of the dose disposed by the various routes were not significantly changed in this population. For the diabetic group, however, although there was no significant change in either \( S_G \) and \( S_I \), explicit incorporation of the urinary loss significantly decreased the percentage of the dose utilized by the three metabolic pathways combined (\( P < 0.05 \)). Of particular importance was the decrease in the total fraction of the dose utilized in this group, which decreased from 91 ± 1 % estimated from the standard model to 81 ± 2 % once the excretion route had been added.

In conclusion, the present study has indicated that measurement of the appearance in body water of label derived from the dose given in IVGTT experiments is a useful supplement to the protocol for minimal model studies. Analysis of the combined data may be useful for assessing glycogenesis in skeletal muscle.

**APPENDIX**

**Two-compartment minimal model of plasma glucose metabolism**

The two-compartment minimal model assumes that (i) insulin-independent glucose disposal occurs from the accessible glucose pool \((G_1)\), via two pathways, one at a constant rate \((U_1)\) and the other first order in glucose mass \([U_1'(t)]\), and (ii) insulin-dependent glucose disposal \([U_2(t)]\) occurs only from a slowly exchanging non-accessible pool \((G_2)\) and is parametrically controlled by insulin in a compartment remote from the plasma. During an IVGTT, plasma glucose usually exceeds the renal threshold for part of the time. We therefore include a further mechanism for glucose disposal: (iii) when the renal threshold is exceeded, losses from the accessible pool occur due to saturation of tubular reabsorption. This pathway is denoted \( U_2'(t) \).

Consideration of the exchange between compartments, and the production \([P_2(t)]\) and utilization processes in the steady state (when renal losses do not occur) leads to

\[
P_2(ss) = U_1 + U_1'(ss) + k_{12}G_1(ss) - k_{12}G_2(ss) \]

\[
U_2(ss) + k_{12}G_2(ss) = k_{21}G_1(ss) \]

where ss is the steady state, and \( k_{12} \) and \( k_{21} \) are fractional rate constants of exchange between \( G_1 \) and \( G_2 \). Assumption (ii) generates the concept of insulin action, denoted \( Y(t) \), where \( U_2(t) = Y(t)G_2(t) \), which is related to plasma insulin by

\[
\frac{dY(t)}{dt} = K_1[I(t) - Y(t)] \]

where \( k_{21} \) is fractional rate constant of irreversible loss of insulin from the remote compartment, \( K_1 \) is the composite fractional rate constant of appearance of insulin action and \( I(t) \) is plasma insulin as a function of time.

In the basal state, \( U_2(ss) = k_{12}G_2(ss)I(ss) \), where \( I(ss) \) is the steady state of plasma insulin, which is combined with eqn (1) to give

\[
U_2(ss) = \left( \frac{k_{21}K_1I(ss)}{k_{12} + K_1I(ss)} \right) G_1(ss) \]

Two constraints now need to be applied to ensure that a unique fit of experimental data to the model can be made. The most recent proposals [1] are (i) that the constant term \( U_1 \) be a fixed fraction, \( \alpha \), of total glucose utilization in the steady state, and (ii) in the steady state, the total rate of insulin-independent utilization be a fixed multiple, \( \beta \), of the insulin-dependent rate. From which

\[
U_1 = \alpha(\beta + 1)U_2(ss) \]

\[
= \alpha(\beta + 1) \left( \frac{k_{21}K_1I(ss)}{k_{12} + K_1I(ss)} \right) G_1(ss) \]

\[
U_2'(ss) = [\beta - \alpha(\beta + 1)]U_2(ss) \]

\[
= [\beta - \alpha(\beta + 1)] \left( \frac{k_{21}K_1I(ss)}{k_{12} + K_1I(ss)} \right) G_1(ss) \]

\[ \]

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The general (non-steady-state) expressions for the four pathways are written

\[ U_1(t) = U_1 = \alpha(\beta + 1) \left( \frac{k_{21} K_I I(s)}{k_{12} + K_I I(s)} \right) G_1(s) \]

\[ U'_1(t) = U'_1 G_1(t) G_1(s) = \beta - \alpha(\beta + 1) \]

\[ \times \left( \frac{k_{21} K_I I(s)}{k_{12} + K_I I(s)} \right) G_1(t) \]  \hspace{1cm} (5)

\[ U''_1(t) = U'_1 \left( \frac{F_G}{V_1} (G_1(t) - V_1 H) \right) \quad G_1(t) \leq V_1 H \]

\[ U''_1(t) = U'_1 \left( \frac{F_G}{V_1} G_1(t) \right) \quad G_1(t) > V_1 H \]

\[ U_2(t) = Y(t) G_2(t) \]

where \( V_1 \) is the distribution volume of the accessible pool, \( F_G \) the glomerular filtration rate, and \( H \) the renal threshold. Adopting the usual definitions of \( S_G \) and \( S_I \)

\[ S_G = \frac{d(U_1(s) + U_2(s))}{dG_1(s)} \]

\[ = (\beta + 1)(1 - \alpha) \left( \frac{k_{21} K_I I(s)}{k_{12} + K_I I(s)} \right) \]  \hspace{1cm} (6)

\[ S_I = \left( \frac{k_{21} k_{22} K}{k_{12} + K_I I(s)} \right)^2 \]  \hspace{1cm} (7)

expressions for the insulin-independent clearance routes are

\[ U_1(t) = \alpha(\beta + 1) G_1(s) S_G \]

\[ U'_1(t) = \beta - \alpha(\beta + 1) \frac{(\beta + 1)(1 - \alpha) S_G G_1(t)}{G_1(t)} \]  \hspace{1cm} (8)

The production in the basal state is given by

\[ P_1(s) = \frac{S_G G_1(s)}{(1 - \alpha)} \]

and, as FCPR (fractional plasma clearance rate) under steady-state conditions [FCPR(s)] is equal to the production rate divided by the plasma glucose mass, the current assumptions force FCPR(s) to be a fixed multiple of \( S_G \) (in contrast with the original two-compartment model [8]).

By the principles of tracer/tracee indistinguishability, the equations analogous to eqn (4) for tracer material are found to be

\[ U''_1(t) = U'_1 \left( \frac{F_G}{V_1} G_1(t) - V_1 H \right) \quad G_1(t) \leq V_1 H \]

\[ U''_1(t) = U'_1 \left( \frac{F_G}{V_1} G_1(t) \right) \quad G_1(t) > V_1 H \]

\[ U_2(t) = Y G_2(t) \]

where * denotes parameters associated with tracer material. From the eqns (5) and (9) it is possible to calculate the following:

- Total quantity of glucose utilised from the plasma via the zeroth order route during the test
- Total quantity of glucose utilised from the plasma via the first order route during the test
- Total quantity of the dose lost to the urine (note the integrals are evaluated between \( t_1 \) and \( t_2 \), the period during which the renal threshold is exceeded)
- Total quantity of the dose utilised from the plasma via the zeroth order route
- Total quantity of the dose utilised from the plasma via the first order route
- Total quantity of the dose utilised from the remote compartment

where \( T \) is the duration of the test, \( D \) and \( d \) are the total glucose dose and the quantity of label given respectively. \( H \), the renal threshold, was taken as 10 mmol [29], whereas \( F_G \), the glomerular filtration rate, calculated from the Cockcroft–Gault equation [30]

\[ F_G = \frac{(140 - \text{age}) \cdot \text{weight}}{1200}, \text{dm}^3 \cdot \text{h}^{-1} \]

in which we have taken a representative value of 10 mg·dm<sup>-3</sup> for the concentration of plasma creatinine.

The parameters \( \alpha \) and \( \beta \) are believed to depend on physiological status. The values adopted were derived from a single study [31] in which it was found that there is an enhanced zeroth-order element (\( R_{d,2} \)), but decreased mass action effect in diabetes, which will consequently be a feature of the interpretation of all investigations using this model. Since the introduction of the two-compartment model, it has been customary to adopt a value of \( \beta = 1 \) [8] but, in order to maintain physiological plausibility, a value of \( \beta = 5 \) has been adopted for subjects with diabetes [1], although there is no experimental evidence to support this choice. We have briefly examined the effects of varying \( \alpha \) and \( \beta \) and have found that increasing \( \alpha \) causes a marked decrease, and increasing \( \beta \) gives a slight increase in the fitted \( S_G \); however, total fraction of the dose taken up is robust to changes in these parameters, being changed by approx. 1% across their expected ranges.

**Model fitting**

Modelling was accomplished using a spreadsheet package (Microsoft Excel). A simple iterative method was chosen to maximize the flexibility in developing the calculations. Euler’s method was used to approximate the time courses from their derivatives, and compared with plasma concentrations scaled by the distribution volume, which
is one of the five independent parameters of the fit along with $k_{Rd}$, $k_{21}$, $k_{21}$ and $K_i$.

The initial conditions were taken to be $G_1(0)=d$, $G_2(0)=0$, and $Y^{*}(0)=K_i/Y(0)$. The tabulated values of $R(t)$, $G_1(t)$, $G_2(t)$, and $Y^{*}(t)$ produced by this method are readily used for the calculations of the integrals needed for the quantities utilized by the various routes.

Fitting of the model parameters to experimental data was accomplished by minimizing the sum of the squared residuals of the observed and modelled plasma-labelled glucose concentrations using the built-in non-linear optimization function of the spreadsheet.

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Received 28 October 2003/30 January 2004; accepted 4 February 2004
Published as Immediate Publication 4 February 2004, DOI 10.1042/CS20030353