Diabetologia (2002) 45:1053–1084
DOI 10.1007/s00125-002-0841-6

Diabetologia

*Reviews*

# Quantitation of basal endogenous glucose production in Type II diabetes

## *Importance of the volume of distribution*

**J. Radziuk, S. Pye**

Diabetes and Metabolism Research Unit, Ottawa Hospital, Ottawa Health Research Institute and University of Ottawa, Ottawa, Canada

## Abstract

The rate of endogenous glucose production (*EGP*) is important in understanding the pathophysiology of Type II (non-insulin-dependent) diabetes mellitus, the aetiology of its complications, and the identification of potential therapeutic targets. A great deal of effort has therefore been expended in its evaluation. Most measurements in humans have been made using tracers, or labelled analogues of glucose. Experimental strategies have included the injection and the infusion of such tracers which were often primed to achieve constant concentrations of the label more quickly. Primers have either been fixed or adjusted to the ambient glycaemia in each diabetic subject. Analyses were carried out using steady-state or non-steady-state calculations, the latter based on a one-compartment model or higher order systems. The principal finding of this review is that all approaches yield the same *EGP* when an appropriate model of the system is used. Under basal conditions, a single compartment model is sufficient to evaluate *EGP*, but the estimation of the volume of distribution, V, from individual data is critical in obtaining consistent results. Other sources of variation arose from the length of the fasting period and the patient population being studied. Overall, in Type II diabetes, *EGP* is frequently high in the morning and decreases gradually to rates comparable to healthy control subjects. This can be a very delayed response to a preceding meal, but more likely corresponds to an accentuated circadian rhythm in glucose production. Metabolic clearance of glucose, on the other hand, is decreased in diabetes, and remains so during the course of the day. [Diabetologia (2002) 45:1053–1084]

**Keywords** Glucose production, tracer kinetics, Type II diabetes, mathematical models, metabolic clearance rates, circadian rhythms.

Received: 26 November 2001 / Revised: 27 February 2002
Published online: 3 August 2002


*Corresponding author:* J. Radziuk, MD, PhD, Ottawa Hospital (Civic Campus), 1053 Carling Ave, Ottawa, Ontario, Canada, K1Y 4E9, E-mail: jradziuk@ohri.ca

*Abbreviations:* DM2, Type 2 diabetes mellitus, non-insulin-dependent diabetes; *EGP*, endogenous glucose production rate of glucose; *MCR*, metabolic clearance rate of glucose; *M*, *M**, mass of glucose and tracer in system respectively; *I**, amount of tracer injected; $R_a$, rate of glucose production or appearance (=*EGP*); $R_a$*, rate of tracer appearance or infusion; $R_d$, rate of (whole body) glucose disappearance, utilisation, or uptake; $R_d$*, rate of (whole body) tracer disappearance or uptake; $C$,$C_1$, glucose concentration in sampled (plasma) compartment ($C_1$, is for multiple compartments); $C$*,$C_1$*, tracer concentration in sampled compartment ($C_1$*, is for multiple compartments); $a$,$a_1$, specific activity or enrichment in sampled compartment; $k_{12}$,$k_{21}$, exchange parameters between compartment 1 and 2 of 2-compartment model; $V$,$V_1$, volume of distribution of sampled compartment; $k$, fractional disappearance rate; $p$, pool fraction; $V_D$, volume of distribution as fraction of body weight.

## Introduction

Insulin resistance is the hallmark of Type II (non-insulin dependent) diabetes mellitus and indeed of syndrome X or the "insulin resistance syndrome". During the course of the early development of the disease however, this resistance remains largely compensated resulting in the hyperinsulinaemia which is often considered as its reliable measure. When this compensatory mechanism fails, glucose intolerance, hyperglycaemia and the overt manifestations of clinical diabetes supervene (e.g. [1]). It has long been hypothesized that the fasting hyperglycaemia was dependent to a large extent on an increase in glucose production. Important correlations between fasting glucose and glucose production were found in a number of studies [1, 2, 3, 4, 5, 6, 7, 8, 9]. Similarly, its inadequate suppression during meals could contribute considerably to glucose intolerance [9, 10, 11]. Impaired hepatic uptake would also increase net splanchnic production [1, 9, 11, 13]. Nevertheless, other investigators [14, 15, 16, 17, 18, 19] concluded that glucose production is altered only marginally during fasting hyperglycaemia and changes substantively only when there is a high increase of glucose concentrations. The discussion centred largely on the methodology.

In almost all cases, the rate of glucose production was measured using tracer methods. To accomplish this, a tracer analogue of glucose was administered in various ways, during the course of a study. Studies could be divided into those that were basal and those where major perturbations, such as meals, hormone administration, or exercise, occurred. The latter could be termed 'non-basal'. Any perturbation induced in the course of a study, usually follows a period where basal measurements are made. In normal subjects, the basal state is synonymous with steady-state conditions. Since these two terms do not refer to the same conditions, it would be useful to have definitions. 'Basal conditions' are those which prevail at rest, and when no 'exogenous' perturbation (e.g. meal) is imposed on the system. 'Steady-state' conditions are present when metabolite concentrations, as well as their fluxes, are all constant. They thus describe 'endogenously' generated conditions, where homeostatic mechanisms maintain the metabolic 'set-points'. They occur between those states that can be characterized as responses to perturbations and usually follow a period of rest and fasting. In diabetes, and in other states of metabolic dysregulation, basal conditions might not necessarily correspond to a steady-state. This can easily be seen in patients with Type II diabetes, where glycaemia frequently continues to decrease during the day, after an overnight fast [1, 3, 4, 20, 21, 22, 23]. In diabetes, therefore, the responses to perturbations can be characterized as highly extended in time and are symptomatic of the impairments in homeostatic mechanisms.

Most tracer study designs include a basal period during which tracer is infused at a constant rate. Almost always, it is 'primed' with an injection of the same tracer at the start of the infusion with the aim of achieving a near-constant tracer concentration. Under completely steady-state conditions, the specific activity (or enrichment) of glucose ($a$, the ratio of plasma tracer and glucose concentrations) will also be constant and the production rate of glucose is calculated simply as $R_a^*/a$, where $R_a^*$ is the rate of tracer infusion, sometimes using a single sample [24]. Although in the normal subject, the constancy of the tracer concentration and its specific activity are synonymous since fasting plasma glucose concentrations are constant, this is no longer true in (Type II) diabetes where plasma glucose concentrations can take a very long time to stabilize after a perturbation such as a meal or an oral glucose load. The general non-steady-state or perturbation problem is necessarily more complex since large changes in metabolite concentrations can be rapidly induced both in time and in space (of metabolite distribution).

Two approaches to solving non-steady-state problems (both basal and non-basal) have evolved. The first is theoretical: a mathematical description of the system is sought which will be adequate for the solution of the flux-determination problem. The second is experimental: the mathematical solutions are simplified by experimental strategies of tracer administration which eliminate the necessity of an elaborate mathematical model. These well-known solutions include the use of primers under basal conditions and the maintenance of a constant specific activity (tracer: metabolite concentration ratio) in the non-steady-state problem. It should be noted that these two experimental approaches address similar issues, but are not, in general, equivalent.

Therefore it is likely that the differences in some of the calculations in the literature could arise from the methods by which these calculations were made and the validity of the assumptions on which they were based (we have already seen three such potential issues: basal versus steady-state, primer versus constant specific activity, theoretical models versus experimental strategies). It is the purpose of this review to examine both aspects of the issue, specifically under 'basal' conditions. These are simpler but do illustrate many of the problems where fluxes change more rapidly.

## The measurement of endogenous glucose production (*EGP*) under basal conditions: tracer methods

### *Balance techniques and the splanchnic bed*

As an introduction to tracer concepts, let us first consider the direct measurement of glucose fluxes across

the liver or the splanchnic bed. Although the kidney [27, 28, 29, 30] and the gut [31, 32] may contribute importantly to *EGP*, this is often considered to take place primarily in the liver [25, 26, 33, 34]. Net glucose production across the liver is directly calculated as the difference between the efflux of glucose from the liver and the influx to the liver [35]:

$$\begin{aligned} &Net\ Glucose\ Production = Efflux - Influx \\ &where \\ &Efflux = Hepatic\ Venous\ Glucose\ Concentration \\ &\qquad \cdot Hepatic\ Blood\ Flow \\ &and \\ &Influx = Portal\ Glucose\ Concentration \\ &\qquad \cdot Portal\ Blood\ Flow \\ &\qquad + Arterial\ Glucose\ Concentration \\ &\qquad \cdot Hepatic\ Arterial\ Blood\ Flow \end{aligned} \tag{1}$$

Hepatic blood flow can be assessed by dye dilution techniques [36] or the use of flow meters. This approach yields unambiguous estimates of net hepatic glucose production. However the liver can simultaneously produce and utilise glucose (e.g. [37]), at least partly because of metabolic zonation within the organ [38]. In order to measure 'total' hepatic glucose production, glucose tracer can be infused and its uptake by the liver measured [37]. Total production would then equal net production added to uptake. In humans this approach is necessarily invasive and under normal circumstances only splanchnic glucose production can be measured [13, 39, 40, 41, 42]. This interposes the gut with its potentially large utilisation and supply (during meals), of glucose. Glucose utilisation, in this case by the entire splanchnic bed, can again be accounted for using tracers. Of note, the potentially larger contributions of the kidney [27, 28, 30] and gut [32] to glucose production were seen mainly because an uptake of glucose nearly equivalent to its production by this organ, was accounted for using tracers.

Three aspects of the arteriovenous measurements are important. This technique when applied simply as arterio-venous differences, is considered accurate only in steady-state conditions [43]. From a whole-body perspective, it does not include the potential production of glucose by other organs such as the kidney [27, 28, 29, 30], and possibly the gut [31, 32]. Any uptake of glucose by the liver and the splanchnic bed, needs to be assessed separately using tracers.

### *Systemic production of glucose*

Accounting for glucose uptake using tracers can be extrapolated to the whole body. Direct measurement across organs is replaced by the tracer dilution principle and a mathematical description of the distribution of glucose throughout the body.

### *Tracers*

Tracers are chemically and metabolically identical to the parent molecule (the 'tracee'), but have been isotopically labelled and are therefore distinguishable from a measurement perspective. Since they are metabolically the same, tracer and tracee (glucose) uptake by tissues are proportional to their concentrations in the immediate environment of these tissues. This is the cardinal principle of the tracer method and can be stated mathematically as:

$$\frac{R_d^*}{R_d} = \frac{C^*}{C} \tag{2}$$

where $R_d$ and $R_d$* are the tissue uptake of glucose and tracer respectively and $C$ and $C$*, the respective concentrations. Only isotopic analogues of glucose are likely to qualify as appropriate tracers, as chemical analogues such as 2-deoxyglucose and 3-O-methylglucose cannot, a priori, be assumed to follow the same kinetics as glucose. It should be noted however that they can be used creatively in quantifying the metabolic fate of glucose molecules [44, 45]. Even in the case of isotopic tracers, care must be taken to use a tracer which tracks the flux that is of interest. Examples of commonly used tracers are given in Fig. 1 where they are also discussed adjacent to the reactions where the label is lost from the molecule. Interpretation of the data hinges on these sites: there are primarily three such loci-phosphoglucose and triose phosphate isomerases and the phosphoenolpyruvate / TCA cycles (1–3 in Fig. 1). Depending on the tracer used, carbon fluxes through the glucose or the fructose cycle are therefore not included in the estimate of *EGP*. Alternately one or both are included. A more detailed description of the different tracers can be found, for example, in [61]. However, it is generally held that [U-$^{13}$C]glucose or glucose labelled with either tritium or deuterium in the 6$^{th}$ or 3$^{rd}$ positions will provide the most consistent estimates of total endogenous glucose production.

### *Mathematical description of the distribution of glucose*

The overall behaviour of glucose within the whole body can be characterized by the law of the conservation of mass. More rigorously, defining the mass of glucose in the system as $M$, its rate of change is described by:

$$\frac{dM}{dt} = R_a - R_d \tag{3}$$

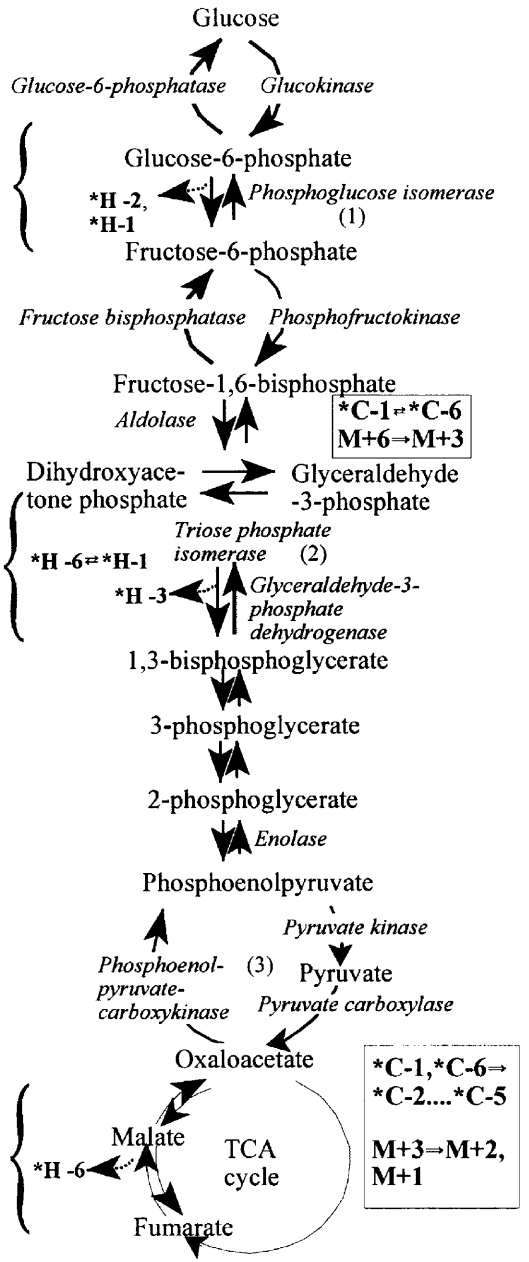
where $R_a$ is the total glucose production and $R_d$ is now its removal from the entire system. A similar equation will define the dynamics of the tracer in the system:

$$\frac{dM^*}{dt} = R_a^* - R_d^* \tag{4}$$

**[2-$^3$H] or [2-$^2$H]glucose**:
is detritiated or de-deuterated at site (1) [46]. This is not of consequence in peripheral tissues but is important in liver since tracer which has lost its label can be dephosphorylated and re-released as unlabeled glucose. This might not be an appropriate tracer for the determination of irreversible liver (or systemic) glucose uptake since it would include some part of the futile cycle (glucose⇆glucose-6-phosphate) in the estimate of the net uptake. Because the equilibration with water might not be complete at the isomerase, a portion of the label might be retained both in the second and the first positions. It is suggested that about 80% of the 'glucose cycle' would be accounted for [47].
*It has been suggested that estimates of EGP would be 17% higher than those using (recycling corrected) [6-$^{14}$C] glucose and 29% higher than those using [3-$^3$H]glucose in normal humans* [48].

**[3-$^3$H]glucose**:
tritium ends up on first position of DHAP. Assuming no redistribution of this tritium to the second position in the isomerase reaction, it will be removed either by exchange with water or in the GA3PDH equilibration. Any tritium that is transfered to the second position will nevertheless be removed at the enolase step. To the extent that this equilibration is complete, [3-$^3$H]glucose will provide an estimate of EGP which includes the 'fructose cycle'**[49]**. It is suggested that 80% of this cycle would be accounted for using this tracer [50].
*EGP estimated using this tracer was found to be about 9% lower (n.s.) than that using corrected [6-$^{14}$C] glucose, suggesting that this cycle is of minimal significance in humans [51]. This is consistent with other recent observations [52] but does not completely agree with previous work which suggested that up to 25% of the EGP determined using [3-$^3$H]glucose could be due to triose phosphate cycling [53]. This was however found to occur in fewer than half the subjects in [54], which compared glucose turnover using [3-$^3$H] and [6-$^3$H] glucose*

**[6-$^3$H]glucose and [6,6-$^2$H$_2$]glucose:**
label is randomized to 1st and 6th position in the aldolase/triose phosphate isomerase reactions. Some of the label on the first position is lost in the phosphoglucose isomerase reaction. A large part of the remainder of the label is lost in the OAA⇆malate⇆fumarate equilibration [55] or the 'phosphoenolpyruvate cycle'.This loss is also not complete [47] so that at most 10% of the label can be recycled back to glucose. Since the [6,6-$^2$H$_2$]glucose can be measured as the M+2 isotopomer, and one of the $^2$H would be lost in the above reactions, negligible label recycling might be expected for this tracer.
*This would lead to an equivalent underestimation (~10%) of EGP for the tritiated tracer*




**[1-$^{14}$C]glucose or [6-$^{14}$C]glucose**
**[1-$^{13}$C]glucose or [6-$^{13}$C]glucose**:
Carbon label is not lost in the process of glycolysis or gluconeogenesis. It is, however, redistributed to other positions. Label in the first position will appear in the sixth, as a result of the triose phosphate isomerase reaction - and vice versa. Label will also be further redistributed as a result of exchange with tricarboxylic acid cycle carbon in the oxaloacetate / malate pool. Assuming symmetry, the infused label in the first and sixth position can be corrected for recycling by subtracting what has been redistributed to the other position. If both measurements are not made, than estimates can be made by comparing total label to that in one of these positions [56]. It is usually assumed that approximately four times the label in the complementary position is recycled. More complex calculations can also be implemented [eg 57,58] if more knowledge of this exchange is desired. Isotopomer analysis [59,60] can be applied to $^{13}$C data for the same purposes. This can also be used to obtain optimal estimates of unrecycled label concentrations.
*When properly corrected, this type of estimate should yield rates of EGP which are near the 'actual' rate - that is, not including any 'futile' cycles. Estimates of the Cori cycle obtained using the redistribution of carbon on these tracers, ranged from 15% in normal subjects to 30% in Type II diabetes*

**[U-$^{13}$C]glucose**:
When the M+6 isotopomer is measured, the EGP calculated will not include any recycled glucose. This is because when small amounts of the tracer (<5% of Ra) are administered, the likelihood of recombination to the M+6 moiety is very small. Thus no cycling through 3-carbon pools will be included in these estimates.
*The tracer is thus equivalent to [6,6-$^2$H$_2$]glucose (measured as M+2), [1-*C]glucose or [6-*C]glucose, when the latter are appropriately corrected for recycling, and to [3-*H]glucose. If the M+6 and the M+3 isotopomers are both considered, the label would be nearly equivalent to [$^3$H-6]glucose*

**[$^2$H$_4$-2,3,6,6]glucose**
This tracer will lose its labels by the schemes already outlined. When measured as the M+4 isotopomer, it will behave as [$^2$H-2]glucose since the label in the second position will be lost the most quickly

where M* is the mass of tracer, $R_d$* is its irreversible removal and $R_a$* is now the rate of tracer administration (for example, a constant infusion).

Except under unusual circumstances however, it is not the total mass of glucose and tracer that are measured, but the concentration at a specific point or within a certain pool. Neither the glucose nor the tracer concentrations are likely to be constant throughout the body. Moreover, the physical distribution of these concentrations will not usually be the same. How these non-uniformities are dealt with, either mathematically or experimentally, will determine the accuracy of the estimates of glucose flux rates, and specifically of its production.

### *The steady state*

The metabolic (glucose) steady-state is characterized by both a constant glucose concentration and constant underlying fluxes. Under steady state conditions, $dM/dt$=0 and therefore, from equation (3), $R_a$=$R_d$.

*Tracer infusions.* In tracer steady-state, although this is not necessary for the metabolic steady state, we again have

$$dM^*/dt = 0 \quad \text{and} \quad R_a^* = R_d^*$$

The important formula for glucose production can then be derived very simply from these steady-state conditions and equation (2):

$$R_a = R_a^* \cdot \frac{C}{C^*} \quad \text{or} \quad R_a = MCR \cdot C \qquad (5)$$

$$\text{where} \quad MCR = \frac{R_a^*}{C^*}$$

$$\text{or} \quad R_a = \frac{R_a^*}{a} \qquad (6)$$

where $a$ is the specific activity defined by $C^*/C$. It should be noted that when $R_a$=$R_d$, this single rate can also be described as the 'turnover rate'. Equation (6) is the formula which is exact under conditions of glucose and tracer steady-state, is frequently applicable, and widely applied to the calculation of the glucose production rate under basal or fasting conditions.

More precisely, equation (6) is exact when tracer mass is negligible, which generally means that the tracer is radioactive. Stable isotopic tracers are however, not massless and their rate of infusion must be subtracted from the calculated $R_a$ in equation (6). The equation then becomes:

$$R_a = \frac{R_a^*}{a} - R_a^* = \frac{(1-a)}{a} \cdot R_a^* \qquad (6a)$$

where $a$ is now the enrichment. It will yield a correction of 1 to 5% depending on the rate of tracer infusion relative to its endogenous production. It should be noted that this subtraction is implied in all subsequent descriptions.

*Tracer injections.* Under steady-state conditions, tracer injections are also frequently used to assess basal turnover rates. The same considerations of conservation of (tracer) mass as were used above, yield the formulas (Appendix A):

$$MCR = \frac{Amount\ of\ tracer\ injected}{area\ under\ the\ plasma\ tracer\ decay\ curve} \qquad (5a)$$

$$and \quad R_a = MCR \cdot C$$

From the derivations, it is clear that the above formulas for *MCR* (equations 5 and 5a) apply to all situations where the *MCR* is constant.

### *The non-steady-state*

Broadly, the non-steady-state is defined by equations (3) and (4). Figure 6 illustrates a generalized concentration distribution and one- and two-compartment models which are special cases of this distribution. Appendix B provides some details on the equations and solutions of these problems. Here we summarise the formulae for the solutions which are relevant to further development:

$$steady\ state: \ R_a = \frac{R_a^*}{a} \qquad (6)$$

$$One\ compt.: \ R_a = \frac{R_a^*}{a} - \frac{V \cdot C}{a} \cdot \frac{da}{dt} \qquad (7)$$

$$Two\ compt.:$$

$$R_a = \frac{R_a^*}{a_1} - \frac{V_1 C_1}{a_1} \cdot \frac{da_1}{dt} + V_1 k_{12} k_{21} \int_0^t e^{-k_{12}(t-\tau)} \left[ \frac{C_1^*(\tau)}{a_1(t)} - C_1(\tau) \right] d\tau \qquad (8)$$

Most variables and parameters have been defined before. In equation (8), the subscript '1' refers to the first (observed or sampled) compartment of a two-compartment system. $k_{12}$ and $k_{21}$ are exchange parameters. Perhaps the most important feature of this group of models, is that each increase in model order (or complexity) provides an additional term, or correction in the formula for glucose production, $R_a$.

## *The one-compartment model and experimental strategies for the assessment of basal rates of glucose production*

Equation (7) describes the well-known equation for the calculation of glucose production rates, which is based on a single compartment model and a uniform glucose concentration throughout this compartment. It

**Fig. 1.** Scheme illustrating the biochemical sites of the loss of label from variously labelled glucose molecules. Literature estimates of relative values of EGP obtained using the different tracers are summarized in italics

provides a correction to the simple formula given by equation (6), and thus could be viewed as compensating for deviations from the steady-state which occur even under basal conditions, in states such as diabetes. In the original formulation [62], a pool fraction, *p*, was used to modify the volume of distribution, which was assumed to be a fixed percentage (20% or 25%) of the body weight. In other words, $V=pV_D$ where $V_D$ was 20–25% of the body weight; *p* was originally estimated as 0.5 [62] and later modified to 0.65 [63]. Rather than modifying a literature estimate of the extracellular volume, we present the developments here with only a single volume of distribution, *V*. Since this volume can often be estimated from the data on an individual patient, it can be considered as the 'effective' volume throughout which glucose is uniformly distributed in that patient and in a specific study. The volume is 'effective' since the glucose concentration is unlikely to be completely uniform and the system is being treated as if it was well-mixed with a concentration equal to that in the circulation.

It is important to note that equation (7) is independent of initial conditions and applies at any time point, during the infusion of tracer at any rate (which could be time-dependent), with the knowledge only of this rate and of the glucose and tracer concentrations and the slope of the specific activity curve at a given time. There are no requirements for tracer steady-state or for constant specific activities. These have been experimentally sought however, since they can simplify calculations, reduce the number of samples necessary, and minimize some types of errors.

It is the contention of this review that any tracer infusion strategy will yield equivalent estimates of the rate of glucose production if the model appropriate to it, is used. Some of the calculations that have been done using a given data set are indeed erroneous. Two ways of circumventing these errors can be envisaged: the use of an appropriate model to analyse the particular data set (the analytical or modelling strategy) or an experimental strategy that diminishes or eliminates the calculation which causes the error. We shall illustrate how both strategies can be employed, and in the course of this development, demonstrate that, with an appropriate analysis, the same answer is obtained for the same situation (fasting hyperglycaemia in Type II diabetes is examined here) for every experimental strategy.

### *Unprimed tracer infusions*

A constant tracer infusion is the experimental protocol most often used, particularly under basal conditions. In normal subjects, when this infusion is continued long enough (often six hours or more), a steady state of tracer concentrations will be reached. Since under basal conditions, glucose is also constant, equation (6), which is a simple ratio of numbers can be used and only a few samples need to be measured. Reaching this steady-state situation more rapidly, is the motivation for priming the system with a preliminary tracer injection and the reason that unprimed infusions are not often used.

However, equations (7) and (8) do not have any requirements for a tracer steady state. Only sufficient tracer data are needed to estimate the parameters describing the system accurately. For a single compartment system, this is only the volume of distribution, *V*. Under conditions where the parameters of the system are all constant [that is the metabolic clearance rate, or *k* in equation (B3) in Appendix B, is also constant], this is equivalent to fitting the tracer data set to the tracer component of equation (B3), or its solution:

$$C^* = \frac{R_a^*}{V \cdot k} \cdot \left(1 - e^{-kt}\right) \tag{9}$$

Recent studies [23], showed that, in fact, *k* was constant under basal conditions even in early diabetes (with plasma glucose concentrations <14 mmol/l) when glucose concentrations are changing, and that equation (9) described the response to an unprimed tracer almost exactly. Both *k* and *V* are estimated in this procedure – hence, the lack of the necessity of estimating *V* independently based on body weight and pool fractions.

### *Primed tracer infusions*

A primer is an injection of tracer administered in conjunction with the initiation of its infusion. It is designed to accelerate the approach to tracer steady state and avoid the use of equations (7) or (9), usually under conditions where glucose steady state already exists. In the context of a true one-compartment system, a perfect primer would achieve instant tracer steady state during a tracer infusion. To see why, let us again consider equation (9), the integrated tracer component of equation (7) when *k* is constant (as it must be in steady-state). At the same time, the response of the tracer concentration to an injection, $I^*$ is:

$$C^* = \frac{I^*}{V} \cdot e^{-kt} \tag{10}$$

Clearly, adding the two responses yields:

$$C^* = \frac{R_a^*}{V \cdot k} \quad when \quad I^* = \frac{R_a^*}{k} \tag{11}$$

If *C* is constant, then so will $C^*/C$ or *a* be. In practice (e.g. [8, 64, 65]), it is the constancy of *a* that has been targeted with the primer, although Hother-Nielsen used the primer specifically to achieve tracer steady-state [22] in subjects with diabetes where it was acknowledged that plasma glucose concentrations were changing appreciably (and *a* would therefore not be constant).

*Fixed primers.* Clearly, to achieve tracer steady-state as rapidly as possible, the dose of primer is critical. The concept of priming was derived in healthy subjects (or in animal models) [62]. The priming dose was usually 100 times $R_a^*$ since $k$ is ~0.01 (equation 11). Several investigations have continued to use such a fixed primer, also in diabetic subjects. It has been suggested that this leads to erroneous calculations of $R_a$ [20, 22]. This is only true because the required calculation, which becomes more complex, is not always used. It is however intrinsically as amenable to a solution as any other strategy. The tracer curve, in a study which is 'overprimed' or 'underprimed' must, in this case, be fitted to the function derived from adding the solutions described in equations (9) and (10):

$$C^* = \frac{R_a^*}{V \cdot k} + \frac{1}{V}\left(I^* - \frac{R_a^*}{k}\right) e^{-kt} \qquad (12)$$

The smaller is the second term of (12), the more rapidly an apparent steady state in $C^*$ is approached. Fitting the data to (12), should however yield equivalent results to the unprimed or perfectly primed approaches, since they are all based on the same model. Again, both $k$ and $V$ can be readily estimated from these data. When the primer is 'perfect', and the system truly well-mixed, equations (11) will hold – and both $k$ and $V$ can still be obtained from the measurements.

*Adjusted primers.* In diabetes, or insulin resistant states, $k$ would be lower than in healthy subjects and therefore a higher primer would be necessary to achieve a rapid steady-state (second term in (12) is ~0). In some studies [20, 21], a fixed higher primer was used in subjects with diabetes. Plasma glucose concentration however, can vary widely in this state. The strategy used by others [22] recognized this by multiplying the average factor of 100 derived above in healthy subjects, by the ratio of the fasting glucose in the diabetic subject with that (5 mmol/l) in healthy subjects.

It is important to re-emphasize that a steady-state of $C^*$ would not have been achievable in either study, had $k$ not been constant and therefore this finding, if it can be further generalized, provides considerable simplification in the calculations. Let us illustrate these concepts in a series of calculations based on data collected for these various approaches.

## Experimental Results

### *Overview*

We have, so far, described a number of experimental and analytical approaches for the measurement of endogenous glucose production which are in general use. There have been many studies in which basal glucose production (and often changes from it) have been evaluated. In particular there has been a great deal of interest in delineating the potential contribution of glucose production to the hyperglycaemia of Type II diabetes. In Table 1, we present a representative sample of such measurements under basal, fasting conditions.

Table 1 shows that there has not been a consensus as to the potential increase in basal glucose production in the diabetic state. Some studies indicate that indeed, such an increase does occur, and *EGP* can even be greatly increased over that in non-diabetic subjects. Others show identical rates of *EGP* in both diabetic and control subjects. A few studies suggest that glucose production can even be decreased. The following are some potential bases for these variations:

### *The (patho)physiological state of study subjects*

*Patient populations.* A number of different patient populations have been used: subjects with mild or severe diabetes, newly-diagnosed or long-standing, obese or non-obese. Subjects had not yet been treated or had been variously treated and with different degrees of intensity. They had been withdrawn from their treatments for varying periods of time. Subjects have often, but not always been studied in the mornings. Measurements have also been made later in the day. To some extent, there has been variability in the period of fasting prior to the measurement.

*Time course of glycaemia.* In a considerable number of, but certainly not all, instances, glycaemia was decreasing during the study. Moreover this could frequently be characterized as a linear decrease. When this occurs, non-steady-state conditions are unequivocally indicated and techniques appropriate for these conditions are needed but not inevitably used.

### *Experimental and analytical approaches*

*Experimental techniques.* A variety of tracers were used. Different protocols were also employed: tracer injection techniques, tracer infusion techniques, a variety of approaches to priming the tracer infusion to achieve tracer steady-state more rapidly. The period of tracer equilibration varied from less than 2 h to more than 10 h. Sometimes samples were taken throughout the infusion period. More often, they were taken over a shorter period (e.g. from 30 to 90 min) after tracer equilibration had occurred or had been presumed to occur. The number of samples varied from 2–3 to 5–6, partly depending on the modelling approach that was intended to be used. Finally, splanchnic balance techniques were used to generate estimates of net production across this bed, without recourse to tracers. On the other hand, tracers were occasionally used to correct for simultaneous splanchnic glucose uptake. In ei-

**Table 1.** A representative selection of calculations of endogenous glucose production in healthy subjects and Type II diabetic subjects

| Reference | Method of calculation | Subjects | Result | | Comments |
|---|---|---|---|---|---|
| Tracer injection | | | | | |
| [66] | Single exponential fit to plasma SA | control<br>DM2 | 11.1±1.1 $\mu k^{-1}$ $m^{-1}$<br>7.8±1.7 | ↓ | [U-$^{14}$C]glucose; *V* 32% and 33% of body weight in control and diabetes |
| [67] | Single exponential fit to plasma activity (formula 10) | non-diabetic<br>DM1 and 2 | 11.1±1.7 $\mu k^{-1}$ $m^{-1}$<br>10.0±5.0 | ↔ | [U-$^{14}$C]glucose |
| [68] | Single exponential fit to plasma activity (formula 10) | non-diabetic<br>DM2 | 15.0±2.2 $\mu k^{-1}$ $m^{-1}$<br>12.8±1.7 | ↔ | [1-$^{14}$C]glucose; plasma activity recycling corrected |
| [69] | Single exponential fit to tracer decay after each injection. Basal appearance reported | non-diabetic<br>DM (nonketotic) | 11.0±0.3 $\mu k^{-1}$ $m^{-1}$<br>14.6±1.8 | ↑ | [U-$^{14}$C]glucose; plasma glucose correlated with $R_a$; vol of distribution ~290 ml/kg |
| [70] | fractional irreversible loss rate using multiple exponential fit to plasma activity (formula A4) | Normal and mild diabetes | Overall: 7.56± 1.06 $\mu k^{-1}$ $m^{-1}$ | ↔ | Plasma glucose correlated negatively with fractional loss rate; no correlation with glucose turnover; [U-$^{14}$C]glucose. |
| [71] | fractional loss rate, *k*, from first 2 h of decay; $R_a=k\cdot$gluc pool size<br>fractional loss rate, *k*, from second 2 h of decay; $R_a$ as above | normal<br>normal (as above) | 678±44 $\mu m^{-1}$<br>583±39 $\mu m^{-1}$ | | [U-$^{14}$C]glucose |
| Tracer infusion | | | | | |
| [71] | SS; 3.5 h tracer infusion | control | 650±44 $\mu m^{-1}$ | | [U-$^{14}$C]glucose; equilibrium level extrapolated tangentially |
| [23] | Non-steady-state analysis: one compartment model. *V* estimated from fit to data | control – 12 h fast<br>control – 22 h fast<br>DM2 – 12 h fast<br>DM2 – 22 h fast | 419±20 $\mu m^{-1}$ $M^{-2}$<br>347±32<br>563±33<br>363±23 | <br><br>↑<br>↔ | [6-$^{3}$H]glucose; metabolic clearance near constant and reduced in diabetes. EGP correlated to glycaemia after 12-h fast and not after 20–22 h fast |
| Primed tracer infusion: fixed primer | | | | | |
| [72] | ST; primer is given as 20 min infusion; early data is used to estimate *V* | control<br>DM2 | 17.2±3.3 $\mu k^{-1}$ $m^{-1}$<br>12.8±2.2 | ↓ | [U-$^{14}$C]glucose; *V* (volume of distribution of rapidly mixing glucose) decreased in diabetes |
| [71] | SS; primer = 10/(1+tT/p) where t: infusion time, T ~ turnover rate; *p* ~ glucose pool size | DM2: morning:<br>evening: | 572±61 $\mu m^{-1}$ $M^{-2}$<br>389±28 | | Glycaemia decreases from 11.9±1.1 to 9.6±0.9 mmol/l from morning to evening in these subjects |
| [73] | SS; primer:tracer inf rate =90–215 – varied according to glucose conc. | control<br>DM2<br>DM2, intensive Rx | 13.9±0.8 $\mu k^{-1}$ $m^{-1}$<br>20.0±2.2<br>12.8±1.7 | <br>↑<br>↔ | [3-$^{3}$H]glucose; 5 sample average of *SA*; intensive insulin treatment; treatment did not affect *MCR* |
| [2] | ST; primer: infusion rate = 100; 2 h tracer equilibration | control<br>DM2 | 7.94±0.44 $\mu k^{-1}$ $m^{-1}$<br>13.0±0.7 | <br>↑ | [3-$^{3}$H]glucose; *EGP* correlated with basal glycaemia |
| [74] | ST; *p*=0.65; *V*=200 ml/kg; primer:infusion rate = 100 | control<br>DM2 | 7.8±0.6 $\mu k^{-1}$ $m^{-1}$<br>10.6±1.1 | <br>↑ | [6-$^{14}$C]glucose; Basal rate of insulin infusion to maintain normoglycaemia. Infusion and levels higher in diabetes |
| [3] | ST; primer:infusion rate = 100; 60 min equilibration + ten 20 min samples | control<br>DM2 | 494±17 $\mu m^{-1}$ $M^{-2}$<br>806±67 | <br>↑ | [3-$^{3}$H]glucose; *EGP* decreased *EGP* and glycaemia correlated from 160 to 128 mg $min^{-1}$ $M^{-2}$ during 200 min in diabetes, parallel to glycaemia; |

**Table 1.** (continued)

| Reference | Method of calculation | Subjects | Result | | Comments |
|---|---|---|---|---|---|
| [4] | ST; primer:infusion rate = 100; *V*=190 ml/kg; *p*=0.65; 5–10 h equilibration | DM2 | 13.2±0.9 $\mu k^{-1}$ $m^{-1}$ | | [3-$^3$H]glucose; *EGP* fell from 2.35 to 1.36 mg·kg$^{-1}$min$^{-1}$ over 10 h, correlated with fall in glycaemia |
| [21] | ST; 18-h tracer infusion starting at 20 h | control 22 h<br>DM2 22 h<br>control 14 h<br>DM2 14 h | 9.4±2.2 $\mu k^{-1}$ $m^{-1}$<br>26.1±2.2<br>9.4±2.8<br>10.0±3.3 | <br>↑<br>↔<br>↔ | [3-$^3$H]glucose; Plasma glucose initially correlated to *EGP*, this correlation disappears by 6 h |
| [20] | ST; 4 h tracer infusion; primer:infusion rate = 100 (control), 150 (diabetes) | control<br>DM2 | 389±2.8 $\mu m^{-1}$ $M^{-2}$<br>411±2.8 | <br>↔ | [3-$^3$H]glucose |
| [6] | SS; 4 h tracer equilibration; primer:infusion rate = 100 | control<br>DM2 | 12.0±0.6 $\mu k^{-1}$ $m^{-1}$<br>22.7±1.0 | <br>↑ | 2 samples for determination of SA; strong correlations among *EGP*, gluconeogenesis, and plasma glucose |
| [75] | SS; 4 h tracer equilibration; primer:infusion rate = 100 | control<br>DM | 11.9±0.3 $\mu k^{-1}$ $m^{-1}$<br>18.2±0.4 | <br>↑ | [2-$^3$H]glucose; 4 samples over ~1–1.5 h with steady state assumed |
| [76] | SS; 6 h tracer equilibration; primer:infusion rate = 100 | control<br>DM2 | 8.9±0.5 $\mu k^{-1}$ $m^{-1}$<br>11.1±0.6 | <br>↑ | ~24 h fasted subjects; percent gluconeogenesis also increased based on nmr measurements |
| [77] | SS; 2.5 h tracer equilibration; primer:infusion rate = 100 | DM2<br>+ metformin Rx | 13.3±1.1 $\mu k^{-1}$ $m^{-1}$<br>11.0±0.7 | | [3-$^3$H]glucose; overweight subjects |
| [18] | ST; 12 h tracer infusion; primer:infusion rate = 100 | control<br>DM-1<br>DM-2<br>DM-3 | 9.39±0.22 $\mu k^{-1}$ $m^{-1}$<br>9.28±0.39<br>11.39±0.39<br>12.11±0.72 | <br>↔<br>↑<br>↑ | [3-$^3$H]glucose; *EGP* correlated with glycaemia; DM-1: [gluc]<10 mmol/l; DM-2: 10–13.9 mmol/l; DM-3: >13.9 mmol/l; |
| [19] | ST; primer: infusion rate = 100; *V*=200 ml/kg; *p*=0.65 | control:<br>DM2: | 458±23 $\mu m^{-1}$ $M^{-2}$<br>989±133 | <br>↑ | [6,6-$^2H_2$]glucose; adjusted primer (below) completely altered results |
| [64] | SS; primer:infusion rate = 100–150; 5–6h equilibration | control:<br>DM2: | 12.0±0.3 $\mu k^{-1}$ $m^{-1}$<br>15.4±0.9 | <br>↑ | [6,6-$^2H_2$]glucose; glycaemia decreasing during study |
| [78] | SS; primer:infusion rate = 81; 4 h equilibration | control<br>DM2 | 433±33 $\mu m^{-1}$ $M^{-2}$<br>767±78 | <br>↑ | [6,6-$^2H_2$]glucose; liver biopsy glucose-6-phosphatase flux also increased in DM2 |
| [11] | ST; *p*=0.65; *V*=250 ml/kg; primer:infusion rate = 120 | control<br>DM2 | overall: ~11 $\mu k^{-1}$ $m^{-1}$ | ↔ | [6-$^3$H]glucose; mild diabetes (fasting glucose 6.7±0.3 mmol/l) |
| [79] | SS; 4 h tracer infusion primer: infusion rate adjusted for fasting glycaemia, 4 samples in last 40 min | control<br>DM2<br>+ metformin Rx | 361±28 $\mu m^{-1}$ $M^{-2}$<br>700±50<br>528±28 | <br>↑<br>↑ | [6 6- $^2H_2$] glucose; new DM2 and treatment-withdrawn |
| [80] | ST; primer: infusion = 100:1 | control<br>DM2 | 422±17 $\mu m^{-1}$ $M^{-2}$<br>478±17 | ↑ | [3-$^3$H]glucose; glycaemia normalized by overnight insulin infusion in diabetic subjects |
| Primed tracer infusion: adjusted primer | | | | | |
| [10] | SS; primer:infusion rate = 100 adjusted for glycaemia | control<br>DM2 | 12.7±0.6 $\mu k^{-1}$ $m^{-1}$<br>13.9±1.3 | <br>↔ | [3-$^3$H]glucose; 120–180 min equilibration; 5 sample SA average; non-obese subjects |
| [81] | SS; primer:infusion rate = 100 adjusted for glycaemia | control<br>DM2<br>DM2 with insulin infusion and normoglycaemia | 10.2±0.1 $\mu k^{-1}$ $m^{-1}$<br>12.1±0.3<br>9.3±0.2 | <br>↑<br>↔ | [3-$^3$H]glucose; strong overall correlation between *EGP* and glycaemia, largely attenuated for [glucose] <8 mmol/l |

**Table 1.** (continued)

| Reference | Method of calculation | Subjects | Result | | Comments |
|---|---|---|---|---|---|
| [2] | ST; primer:infusion rate = 100 adjusted for glycaemia; 6 h tracer infusion; $V$=200 ml/kg; $p$=0.65 | control<br>DM2 | 357±21 $\mu m^{-1}$ $M^{-2}$<br>372±15 | <br>↔ | [3-$^3$H]glucose |
| [82] | One-compt formula (7); primer:infusion rate = 120 adjusted for glycaemia; $V$ calculated from body surface area; 2-h tracer equilibration | control<br>DM2-1<br>DM2-2 | 772±44 $\mu m^{-1}$<br>728±39<br>1038±50 | <br>↔<br>↑ | [6-$^3$H]glucose; obese subjects: DM2-1: [gluc]<7.8 mmol/l (mean 6.5 mmol/l); DM2-2: >7.8 mmol/l (mean 10.8 mmol/l) |
| [8] | One-compt formula (7); primer:infusion rate = 125 adjusted for glycaemia; 4 data points over 45 min after 2–3h equilibration; | control<br>DM2 | 406±17 $\mu m^{-1}$ $M^{-2}$<br>406±11 | <br>↔ | [6-$^3$H]glucose; mild diabetes (fasting glucose: 6.1 mmol/l); Plasma glucose strongly correlated to *EGP* in control and diabetes after 14 and 36–108-h fast |
| [19] | ST; primer:infusion rate = 100 adjusted for glycaemia; $V$=200 ml/kg; $p$=0.65 | control<br>DM2 | 458±23 $\mu m^{-1}$ $M^{-2}$<br>446±80 | <br>↔ | [6,6-$^2H_2$]glucose; glucose conc decreases and enrichment increases with time; ss calculations similar to nss |
| [83] | ST; primer: infusion rate = 100:1 adjusted for glycaemia; 10 h equilibration (22–8h) | control<br>DM2(A)<br>DM2(B) | 9.5±0.3 $\mu k^{-1}$ $m^{-1}$<br>11.1±0.3<br>12.7±0.3 | <br>↑<br>↑ | [3-$^3$H]glucose; SA constant; non-obese subjects with fasting glycaemia <7.8 (A) and >7.8 (B); *EGP* and glycaemia correlated over entire glycaemic range |
| [65] | SS; primer: infusion rate = 100:1 adjusted for glycaemia; 3-h tracer infusion | control (lean)<br>control (obese)<br>DM2 (lean)<br>DM2 (obese) | 12.4±1.4 $\mu^{-1}$ $m^{-1}k_l^{-1}$<br>13.1±0.7<br>16.7±0.9<br>15.2±0.6 | <br><br>↑<br> | [6,6-$^2H_2$]glucose; enrichment constant over period of measurement; glycaemia decreased by 0.05% $min^{-1}$; *EGP* and glycaemia correlated |
| [52] | SS; tracer infusion from 3 to 12 h; primer:infusion rate = 100, adjusted for glycaemia in 3 h expt; 4 samples over 30 min; estimates obtained at 14 and 22 h fast | control (14 h)<br>control (22 h)<br>DM2 (14 h)<br>DM2 (22 h) | 10.0±0.4 $\mu k^{-1}$ $m^{-1}$<br>8.2±0.4<br>10.4±0.3<br>7.6±0.2 | <br><br>↔<br>↔ | [6,6-$^2H_2$]glucose or [3-$^3$H]glucose; no difference in *EGP* with two tracers; Glycaemia decreasing in DM2; Established diabetes (BMI ~30), treated with oral agents +/- insulin |
| [84] | ST; primer: infusion rate = 100:1 adjusted for glycaemia; 2.5 h tracer infusion; multiple samples? | control<br>DM2 (<10)<br>DM2 (>10) | 9.8±0.6 $\mu k^{-1}$ $m^{-1}$<br>8.6±0.8<br>12.6±1.2 | <br>↔<br>↑ | [3-$^3$H]glucose; BMI of patients:29–33; of controls: 27 kg/$m^2$. 16-h fast; diabetes treated, treatment withheld for 3 days; (<10), (>10) refers to fasting glucose (mmol/l); modest correlation of *EGP* with glucose |
| [85] | SS; primer: infusion rate = 100:1 adjusted for glycaemia; 3-h tracer infusion | control<br>DM2 | 12.2±0.7 $\mu^{-1}$ $m^{-1}$ $k_l^{-1}$<br>15.2±0.4 | <br>↑ | [6,6-$^2H_2$]glucose; enrichment constant over period of measurement |
| Splanchnic balance | | | | | |
| [86] | net splanchnic glucose production | control<br>diabetic (ins-treated) | 359±21 $\mu m^{-1}$ $M^{-2}$<br>393±21 | <br>↔ | Some measured arterial-portal differences very small. ~ hepatic glucose production |
| [87] | net splanchnic glucose production | control<br>DM (1 an 2): | 644±50 $\mu m^{-1}$ $M^{-2}$<br>717±83 | <br>↔ | |
| [88] | net splanchnic glucose production | control<br>DM2 | 611±61 $\mu m^{-1}$ $M^{-2}$<br>850±111 | <br>↔ | |

**Table 1.** (continued)

| Reference | Method of calculation | Subjects | Result | Comments |
|---|---|---|---|---|
| [89] | net splanchnic glucose production and based on tracer infusion | control/ balance | 9.8±0.9 $\mu k^{-1}$ $m^{-1}$ | |
| | | DM2/balance | 9.1±0.7 | $\leftrightarrow$ |
| | | control/tracer | 12.1±0.7 | $\uparrow$ |
| | | DM2/tracer | 13.8±0.4 | $\uparrow$ |

Abbreviations used in table: $\mu k^{-1}m^{-1}$: $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $\mu m^{-1} M^{-2}$: $\mu mol \cdot min^{-1} \cdot M^{-2}$; $\mu m^{-1}$: $\mu mol \cdot min^{-1}$; $\mu m^{-1}k_l^{-1}$: $\mu mol \cdot min^{-1} \cdot kg_{ffm}^{-1}$ and ffm is fat free mass. SS: steady-state formula (6); ST: Steele formulation of formula (7). DM2: Type II diabetes; DM1: Type I diabetes

The arrows in the fifth column of the table indicate whether glucose production in the diabetic subjects studied in the particular experiment was increased or remained unchanged relative to the control subjects

ther case, small changes in glucose production occurring in diabetes, could have been obscured by the larger errors inherent in this method, particularly in the absence of a complete steady state and the relative diversity of patients used in these early studies.

*Mathematical models.* In many cases, steady-state approaches were utilised – with an average specific activity and formulas (5, 5a) or (6). In other cases steady-state was not assumed and a one-compartment model of glucose and tracer behaviour assumed (formula 7). In many cases, and necessarily when tracer injections were used, the parameters which determine tracer behaviour were assumed constant – that is, the metabolic clearance, or fractional disappearance rates were considered constant. Sometimes all the parameters of this model (*V, k*) were estimated from the data. At other times, *V* was assumed to correspond to a physical volume of distribution (often modified to account for non-uniform glucose and tracer concentrations).

*Summary*

The variety of results obtained reflects, at least in part, the diversity of subjects and experimental conditions. There is an evolution of glucose dynamics in patients with diabetes over time: this is both acute, such as circadian variations, and chronic, e.g. with the duration of the diabetic state. It is not surprising that different results are obtained in different groups of subjects. On the other hand, in some cases experimental observations could have been made over too short a period of time and perhaps because of this limited horizon, glucose concentrations or specific activities (after primed tracer infusion) were found which were not distinguishable from a constant value. This could in turn, have led to assumptions as to the steady-state nature of the system. Such assumptions or those on the values of system parameters, could generate errors which would also contribute to the variability of *EGP* estimates.

### *A detailed reanalysis*

To understand the potential methodological sources of variance among the results, we present three studies carried out in diabetic subjects [22, 23]. Each study used one of the methods described which is based on tracer infusion, i.e. using an unprimed tracer infusion, a fixed primer, or an adjusted primer, as defined above. All data used here are from these published studies [22, 23]. We show that, when appropriate experimental or analytical techniques are applied, estimates of *EGP* display qualitatively similar characteristics, even in somewhat disparate groups of patients.

*Extended fast; Type II diabetes; unprimed tracer infusion [23]*

After an overnight fast, sixteen subjects, nine with newly diagnosed or diet-treated Type II diabetes and fasting hyperglycaemia and with an overall average BMI of ~35 kg/m$^2$, underwent a further fasting period of 8 to 10 h, during which [6-$^3$H]glucose was infused at a constant rate (data shown in Fig. 2). Plasma glucose concentrations are seen to fall (from 10.7 mmol/l to 6.5 mmol/l at 10 h, or at a rate of 0.42 $mmol \cdot l^{-1} \cdot h^{-1}$) and are smoothed for the purposes of the $R_a$ calculations. The tracer concentrations are fitted to the function described by equation (9). Average fits are also shown. As described [23], the sums of squared differences were small and a runs test showed the randomness of the errors. When some deviation from an optimal fit was evident, *k* was allowed to vary, the deviations, $\Delta k$, were calculated from [23]:

$$\frac{d\Delta C^*}{dt} = -k\Delta C^* - C^*\Delta k \qquad (13)$$

where *k* itself is obtained from equation (9), and the $k+\Delta k$ were used in calculating *MCR*. The resulting *MCR* are also depicted and have no consistent variation from a constant value. The $R_a$, calculated from equation (7) and $R_d$ calculated from equation (3) are also shown. It is evident that, in subjects with

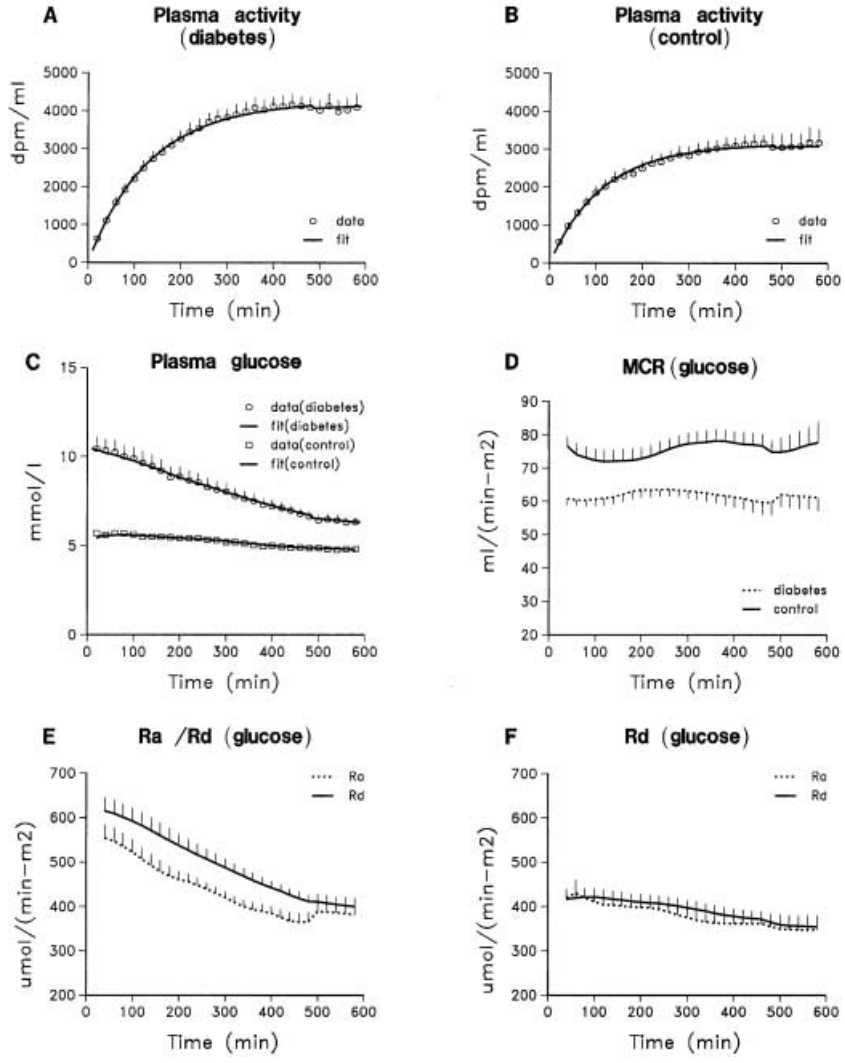



**Fig. 2A–F.** Results of a study with unprimed infusion of tracer in subjects with Type II diabetes and healthy control subjects [23]. **A** Plasma tracer concentrations during a 10-h infusion of [6-$^3$H]glucose in patients with Type II diabetes. The *solid line* represents the best fit to equation (9). **B** The analogous tracer concentrations and fit in control subjects. **C** Plasma glucose concentrations throughout the 10-h experimental period in patients with Type II diabetes and in non-diabetic control subjects. **D** The metabolic clearance rate (MCR) of glucose in diabetic patients and control subjects. **E** Rates of glucose production ($R_a$) and utilisation ($R_d$) for the diabetic patients (Note that two of the subjects failed to complete the last 2 h of the study). **F** Rates of glucose production ($R_a$) and utilisation ($R_d$) for the control subjects. Data are adapted with permission from [23]

Type II diabetes, both fluxes are initially increased ($R_a$~570 µmol min$^{-1}$ m$^{-2}$), show a gradual decrease in time and, in this particular group, reach a rate of glucose production indistinguishable from the constant rate in the non-diabetic control subjects (~350 µmol min$^{-1}$ m$^{-2}$). $R_d$ is consistently higher than $R_a$ and the difference, $R_d$–$R_a$, clearly drives the decline in glucose concentrations. The *MCR* derived from this $R_d$ was lower in diabetes than in the control subjects (62 vs 76 ml min$^{-1}$ m$^{-2}$). The mean volumes of distribution in both cases were estimated at 18% of the body weight, considerably higher than the usual assumption of 13% (e.g. [22]).

*Extended primed tracer infusion; Type II diabetes; fixed primer [22]*

Eleven subjects with Type II diabetes, with treatment withdrawn for at least 2 days, and seven control subjects (overall BMI ~28 kg·min$^{-1}$·m$^{-2}$) underwent a similar overnight fast and a 6 h tracer infusion with an initial injection of tracer primer which was fixed at 100 times the infusion rate. Data were collected at 10 min intervals. Glucose and tracer data (with smoothing fits) are shown (Fig. 3A,C). Again plasma glucose concentrations decreased in diabetes with an average rate of 0.52 mmol·l$^{-1}$·h$^{-1}$, slightly more rapidly than above. $R_a$ was calculated, as originally described [22]: Glucose and tracer were smoothed using the 'optimal segments method' [90]; $R_a$ was calculated from equation (7) and $R_d$ from equation (A3), using the approximations: $C=(C_1+C_2,)/2$, $a=(a_1+a_2)/2$,

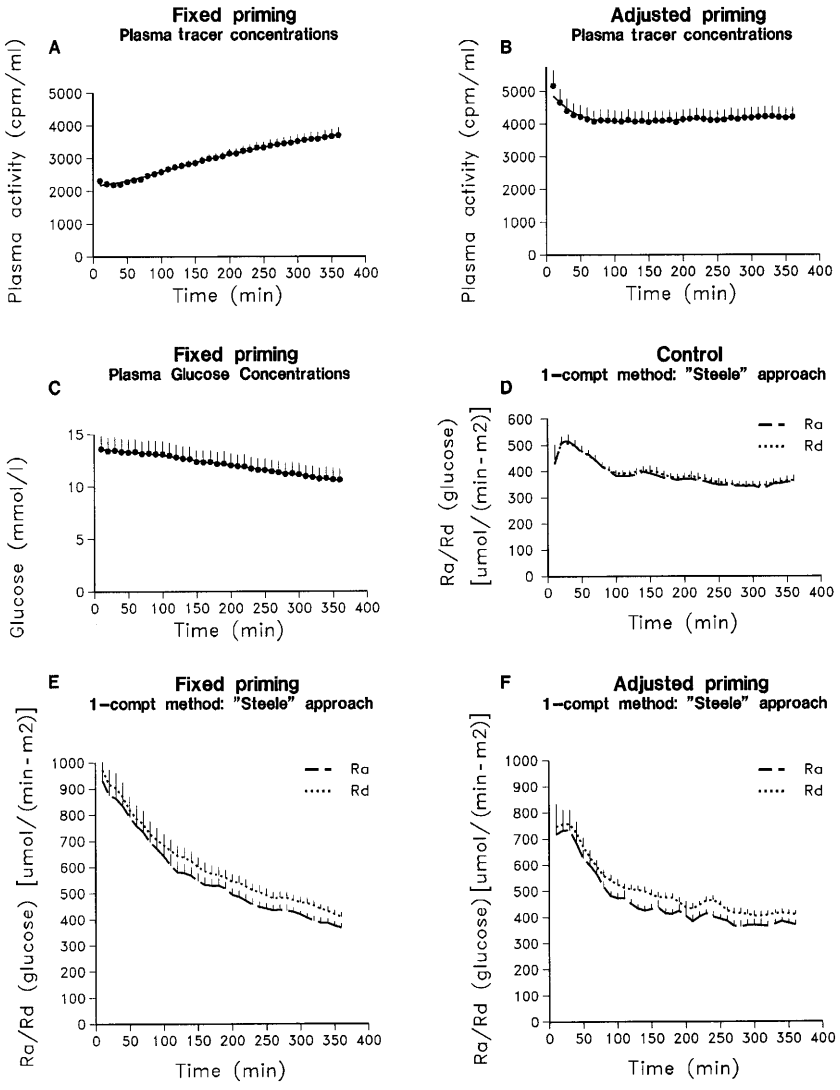



**Fig. 3A–F.** Summary of a reanalysis of studies [22] based on tracer infusions with fixed and adjusted primers in subjects with Type II diabetes. **A** Plasma tracer concentrations (activity) during a 6-h infusion of [3-$^3$H] glucose with a primer equivalent to 100× tracer infusion rate. **B** Data analogous to (**A**) when the primer was adjusted for ambient glycaemia: i.e. primer in (**A**) was multiplied by (measured glucose concentration/5). **C** Plasma glucose concentrations throughout the 6-h experimental period when the fixed primer was used. These were equivalent for the adjusted primer study. **D** Rates of glucose production ($R_a$) and utilisation ($R_d$) for subjects who served as controls for the Type II diabetic patients reported in the remainder of the figure [primer was fixed as in (**B**)]. **E** Rates of glucose production ($R_a$) and utilisation ($R_d$) for the diabetic patients calculated using the 'fixed primer' data and a one-compartment calculation with the volume of distribution equal to $pV_D$ where $p$=0.65 and $V_D$=20% of body weight. **F** Rates of glucose production ($R_a$) and utilisation ($R_d$) for the diabetic patients calculated using the 'adjusted primer' data and the same model as in (**E**)

$dC=C_2-C_1$ and $da=a_2-a_1$ where the subscripts refer to two successive measured values ($dt$=10 *min*). The effective volume of distribution was estimated as $pV_D$ where $p$=0.65 and $V_D$=20% of the body weight.

This calculation yields high initial estimates [22] of the fluxes, $R_a$ and $R_d$ (Fig. 3E). These decrease gradually so that, at 6 h, their value is close to that shown for the control subjects. The control subjects also show an initial increase in $R_a$ and $R_d$, which dissipate within an hour, to a near constant value. The question arises as to whether these variations in $R_a$ and $R_d$ can be explained by physiological considerations or whether they are methodological.

*Primed tracer infusion; adjusted primer [22]*

The following set of studies suggests that the answer to the above question is: at least partly methodological.

An experimental strategy was used to overcome the problem of wide variations in tracer concentrations in time, and among studies, which seem to lead to these initial high estimates of $R_a$. Results are shown in Fig. 3B, F. The same subjects as above received the same tracer infusion, but with a primer which was adjusted to their initial fasting plasma glucose concen-

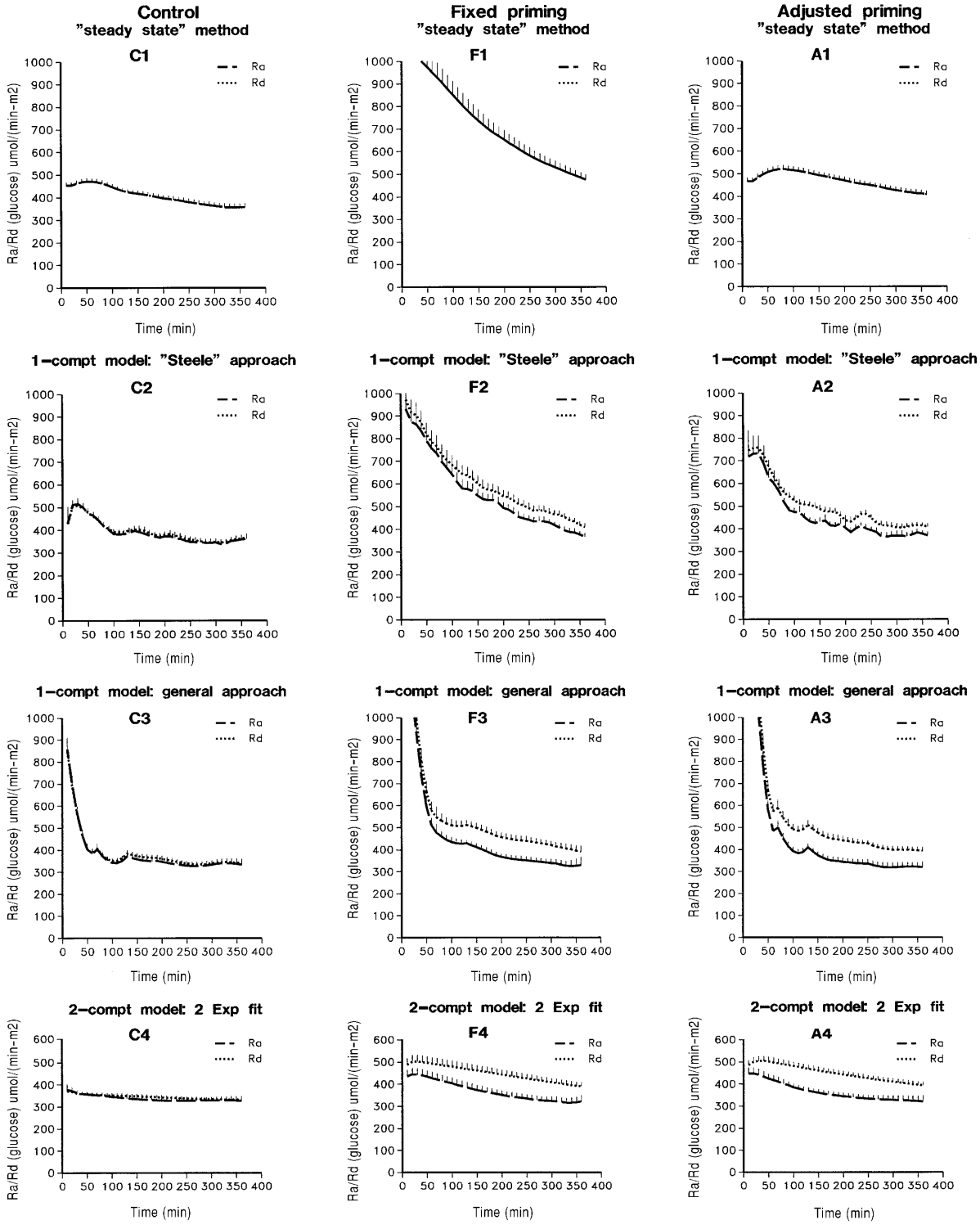



**Fig. 4.** Legend see page 1067

trations (i.e. 100 × subject's fasting plasma glucose/5 mmol·l$^{-1}$). The same measurements and calculations were used as above. It can be seen that the revised priming strategy achieves rapid convergence to essentially constant tracer concentrations (Fig. 3B). This indicates that as in the first series of studies, *MCR* is close to constant. Moreover, Fig. 3F shows that $R_a$ and $R_d$ converge much more rapidly (in less than 100 min) to values which decrease slowly over time, reminiscent of the first study shown in Fig. 1. This suggests that the fixed priming method with simple smoothing of data contains errors which are alleviated either by doing the calculations on the basis of a constant *k* (Fig. 2), or by minimising the variations in the tracer response to its infusion using a more appropriate (adjusted) primer (Fig. 3F).

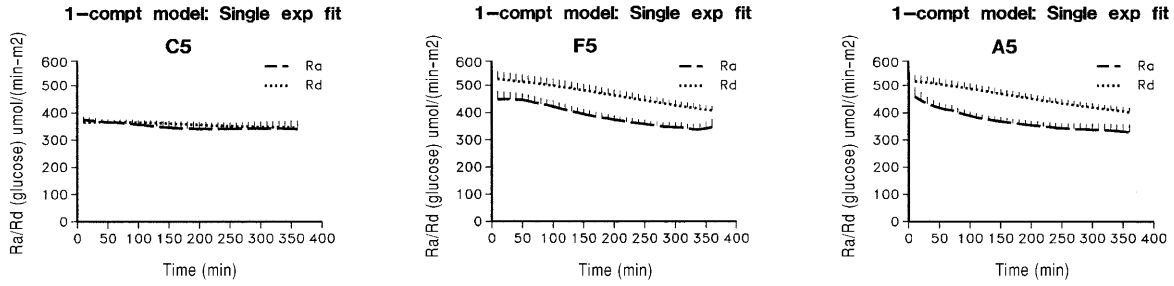



**Fig. 4.** Reanalysis of data in [22] and Fig. 3 to illustrate the convergence of the data to the same quantitative results as experimental and modelling strategies are altered. The raw data (tracer and glucose concentrations) are shown in Fig. 3. The *first column* (designated **C**) represents control studies. The *second column* (designated F) shows studies done in Type II diabetic subjects using a tracer infusion and the fixed primer (see Fig. 3). The *third column* (designated A) shows studies done in the same subjects but using a tracer infusion with an adjusted primer (see Fig. 2). Each row represents a different calculation carried out on each of the three sets of studies. First set of panels (numbered '1'): these show the calculations using the 'steady-state' formula (equation 6). Second set of panels (numbered '2'): the calculations here are done using a one-compartment model (equation 7) with the volume of distribution equal to $pV_D$ where $p$=0.65 and $V_D$=20% of body weight ('Steele' approach). Note the decrease in the calculated fluxes in diabetes when an adjusted primer is used and the tracer concentrations are near constant (Fig. 3). Third set of panels (numbered '3'): the calculations here are done using a one-compartment model (equation 7) with the volume of distribution, $V$, estimated from the tracer data in each subject (equation 12). Note that in panel F3, $R_d$ is now the same as in A2. In panel A3, both $R_d$ and $R_a$ are the same as those in panel F3. Fourth set of panels (numbered '4'): the flux calculations are done using a two-compartment model (equation 8). Note the disappearance of the initial peak in these fluxes. Fifth set of panels (numbered '5'): Calculations are again done using a one-compartment model and estimated $V$ (as in row 3) but with early mixing-related tracer data ignored (see Fig. 5). These are analogous to those in row 4

## Reconciliation of results

Three variants of approaches to the calculation of the rates of glucose production and disappearance have been used. Each yields somewhat different results. This is a matter of concern because each method seems to suggest different conclusions about the behaviour of these fluxes after an overnight fast in Type II diabetic subjects, and therefore the relative potential importance of increases in basal glucose production in the pathogenesis of the disease and as therapeutic targets.

We contend that there is only one physiologically correct answer and the use of the appropriate calculation for a specific experimental situation should yield this answer, independently of the tracer-based strategy. We therefore review the calculations and determine if such considerations can indeed yield equivalent values and time courses for $R_a$ and $R_d$, using all three data sets. If this is true, then our hypothesis is correct. The achievement of identical answers using the diverse data sets would also generate a degree of confidence in the validity of these calculations. The re-analysis, which is illustrated in Figure 4, will simultaneously yield a summary of the potential sources of error in this type of calculation.

For more detailed comparisons, Table 2 provides the numerical summary corresponding to the calculations of $R_a$ in Fig. 4. Equations (6, 7, 8) which have been deliberately grouped together provide the main part of the sequence of these calculations.

### *Basal conditions: steady-state or non-steady-state?*

#### *The 'steady-state' calculation*

The first set of panels in Fig. 4 (C1, F1, A1; $R_{ass}$, Table 2 ) show the results of the 'steady-state' calculation for the two data sets described above, in diabetic subjects as well as in control subjects, (fixed and adjusted primers) using equation (6). This is patently not correct in any of the cases. For control subjects, it would be anticipated that turnover would be closer to constant. For diabetic subjects, the glucose concentration is changing throughout the study in both cases and therefore there is no steady-state. For the fixed primer, the tracer concentration is also changing, and changing rapidly. This leads to a dramatic decrease in apparent glucose production from over 1000 μmol/(min-m$^2$) to a value near 500 μmol/(min-m$^2$) over 6 h. Moreover, by the definition of steady state, there is no difference between $R_a$ and $R_d$, so that there is no difference, $R_d$–$R_a$, which would drive the decrease in glucose concentrations. For the adjusted primer the tracer concentration is nearly constant (Fig. 3) and the estimate of production based on the steady-state formula seems much more defensible. The initial increase however, is due to the primer decay which is present in the first hour of the study. The decrease in $R_a$ thereafter is directly proportional to the decrease in plasma glucose concentration. Again, there is no difference, $R_d$–$R_a$, driving the decrease in concentration.

#### *Taking the non-steady-state into account: the one-compartment calculation (the 'Steele' technique)*

Clearly, to obtain physiologically tenable calculations, non-steady-state approaches must be used. The tradi-

**Table 2.** Rate of glucose appearance in control and diabetic subjects, calculated using different methods

| Time (min) | Panel Fig. 3 | 30 | 60 | 90 | 120 | 150 | 180 | 240 | 300 | 360 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control (fixed primer) | | | | | | | | | | |
| $R_{ass}$ | C1 | 462±14 | 470±16 | 455±15 | 430±13 | 417±14 | 405±15 | 384±16 | 363±16 | 357±17 |
| $R_a$(Steele) | C2 | 516±26 | 465±17 | 402±13 | 383±15 | 392±22 | 371±17 | 353±19 | 345±17 | 361±22 |
| $R_a$(V est'd) | C3 | 564±34 | 383±23 | 352±14 | 352±14 | 359±17 | 350±18 | 332±18 | 334±21 | 333±26 |
| $R_a$(2-exp) | C4 | 363±11 | 354±11 | 348±10 | 341±10 | 335±10 | 330±10 | 328±11 | 329±16 | 327±22 |
| $R_a$(1-exp) | C5 | 369±11 | 364±11 | 358±11 | 351±11 | 346±13 | 342±15 | 341±20 | 342±26 | 339±33 |
| DM2: Fixed primer | | | | | | | | | | |
| $R_{ass}$ | F1 | 1020±81 | 951±72 | 876±64 | 801±58 | 736±52 | 682±46 | 582±35 | 529±30 | 475±25 |
| $R_a$(Steele) | F2 | 863±58 | 758±47 | 670±43 | 580±42 | 547±30 | 529±27 | 447±22 | 418±14 | 368±16 |
| $R_a$(V est'd) | F3 | 925±71 | 511±32 | 444±22 | 426±20 | 411±18 | 383±18 | 353±18 | 338±20 | 329±38 |
| $R_a$(2-exp) | F4 | 443±29 | 427±28 | 409±27 | 390±26 | 373±24 | 359±22 | 337±20 | 323±21 | 322±29 |
| $R_a$(1-exp) | F5 | 449±26 | 441±25 | 427±23 | 410±21 | 394±19 | 381±170 | 360±16 | 346±18 | 346±30 |
| DM2: Adjusted primer | | | | | | | | | | |
| $R_{ass}$ | A1 | 483±17 | 514±17 | 518±18 | 507±17 | 493±16 | 478±17 | 452±16 | 426±16 | 409±16 |
| $R_a$(Steele) | A2 | 732±54 | 598±30 | 481±18 | 451±19 | 431±19 | 412±18 | 401±19 | 368±23 | 371±16 |
| $R_a$(V est'd) | A3 | 1070±106 | 484±25 | 414±19 | 388±18 | 373±17 | 348±19 | 336±19 | 318±22 | 319±24 |
| $R_a$(2-exp) | A4 | 441±26 | 416±22 | 393±18 | 373±16 | 359±16 | 349±16 | 335±17 | 328±21 | 320±27 |
| $R_a$(1-exp) | A5 | 433±26 | 411±20 | 394±17 | 378±15 | 367±16 | 358±17 | 344±19 | 337±23 | 327±30 |

$R_{ass}$ assumes that the system is in steady state (equation 6). $R_a$(Steele) corresponds to a one-compartment formulation of the estimation problem with the volume of distribution corresponding to the extracellular fluid (assumed to be 20% of body weight) multiplied by a pool fraction (0.65) to account for a non-uniform distribution of glucose (ie volume of 13% body weight) (equation 7 with $V=pV_D$). $R_a$(V est'd) is the calculation based on the same one-compartment model above (equation 7) but with V estimated from the data of each individual experiment using the fit to equation (12). $R_a$(2–exp) corresponds to the $R_a$ calculated on the basis of a two-compartment model with the parameters for this model calculated from a two-exponential fit (equations 8, 20 and Appendix B). $R_a$(1–exp) uses a single exponential fit to a restricted data set – the first 30–40 min of data are neglected so that the rapid mixing following the primer injection does not affect the data – this simplification leads back to the simpler approach based on equation 7

tional method, described above, is the formula based on the one-compartment equations (B3), which reduces to equation (7). In this approach, the volume *V*, is usually estimated as $pV_D$, where $V_D$ is a physical volume of distribution corresponding to the extracellular space and *p*, the 'pool fraction', is a factor which compensates for the non-uniform distribution of glucose throughout the system [62, 91]. It is usually considered to be less than one since glucose appears in the circulation, where it is measured, and its concentrations would therefore be highest at this site. $R_d$, the rate of glucose utilisation or disappearance, can then be calculated from:

$$pV_D\frac{dC}{dt} = R_a - R_d \quad or \quad R_d = R_a - pV_D\frac{dC}{dt} \tag{14}$$

This calculation is exemplified by panels C2 and F2 of Fig. 4 (see also Table 2). The calculated $R_d$ is now greater than $R_a$, so that $R_a$–$R_d$ is negative, providing the drive which decreases *C* [for example see the first of the equations (14)]. There is a definite decrease in the calculated $R_a$, which however, continues to appear high, particularly in the first several hours of the study in diabetic subjects (Fig. 4, F2). This is underlined in the control study (Fig. 4, C2), where early $R_a$ are also increased. This suggests a methodological rather than a physiological explanation.

### *Strategic crossroads: experimental or modelling approaches?*

At this point strategies to address the problem diverge. On the one hand, model improvements can be considered (i.e. in the direction of panel F3 of Fig. 4). On the other, the need for these improvements could be obviated by adopting an experimental strategy which simplifies the formulae (i.e. in the direction of panel A2 of Fig. 4). The history of the measurement of basal glucose production in diabetes is the history of the development of these apparently competing measurements – and they are only apparently competing as we hope to convince the reader.

#### *The experimental strategy: Effect of primers*

From Figures 2 and 3, it can be seen that for the fixed rimer, *dC*/dt*, or the slope of the tracer curve, is a large, positive, and variable number in fasting diabetic subjects. To evaluate the impact of this slope on the calculation of $R_a$, we rewrite equation (7) as:

$$R_a = \frac{C}{C^*}\cdot R_a^* + V\cdot\left[\frac{dC}{dt} - \frac{C}{C^*}\cdot\frac{dC^*}{dt}\right] \tag{15}$$

*dC/dt* is negative (Fig. 3), and, in the fixed primer case, *dC*/dt* is positive, so that the second term within

the square brackets of equation (15) is also negative. From the calculations (not shown), the second term of equation (15) can easily be about 50% of the first. It therefore has a major influence on the calculations. In addition, it is multiplied by the volume, *V*. Any error in the estimate of this volume will amplify an inappropriate contribution from the second term. As indicated above, the experimental strategy most often followed to improve the estimates of basal $R_a$, is the administration of a tracer primer which renders $C^*$ nearly constant (the adjusted primer). This would have the overall effect of simplifying equation (15) since it would now only depend on a time varying *C* and *dC/dt*. Thus the largest contribution to the second term of (15) is removed, and the effect of the volume, *V*, on $R_a$ is attenuated. The transition from panel F2 to A2 in Fig. 4, shows the change effected by adding an appropriate primer: the slope of the estimates of $R_a$ and $R_d$ are markedly decreased after about 60 min of the study.

One may also ask, why a primer adjusted according to initial glucose concentrations works as well as it does, in individual subjects. It can, in fact, be shown to have some physiological underpinnings. If one assumes that much of gluco-regulation is geared towards maintaining a sufficient $R_d$ [92], then this could be quantitated as $R_{d(diabetes)}=R_{d(normal)}$. This would imply (since $MCR=Vk$) that:

$$\begin{aligned} MCR_{diabetes}\cdot C_{diabetes} &= MCR_{normal}\cdot C_{normal} \\ &\quad or \\ k_{diabetes}\cdot C_{diabetes} &= k_{normal}\cdot C_{normal} \end{aligned} \tag{16}$$

provided that *V* is the same in each subject, normal, obese or diabetic. Under these circumstances, from equation (11) and (16):

$$I^*_{diabetes} = \frac{R^*_a}{k_{normal}}\cdot\frac{C_{diabetes}}{C_{normal}} = I^*_{normal}\cdot\frac{C_{diabetes}}{C_{normal}} \tag{17}$$

where $I^*_{normal}=100\cdot R_a{}^*$.

### *The modelling strategy: the effect of the volume of distribution, V*

The first step in improving the model is very simple and consists of optimizing the volume of distribution, *V*. The results of this option are shown using the data obtained following the fixed primer of tracer, as one progresses from panel F2 to F3 in Figure 4. A profound decrease in the estimate of $R_a$ occurs, as well as a change in profile. The reason for this is as follows: in the 'Steele' calculation, the volume of distribution, $pV_D$, is imposed on the one compartment model. However, *V* is also one of the two parameters (*V* and *k*) which defines the time course of the tracer concentration, $C^*$, defined by equation (12), during the primed infusion. If *V* is fixed as $pV_D$, this allows only one parameter to vary in optimizing the fit to data which is insufficient. If most of the data (after about 40 min) is fitted to equation (12), estimates of both *k* and *V* are obtained. Using this estimate of *V* in equation (7), the new estimate of $R_a$, shown in panel F3 (Fig. 4), is obtained.

Comparing panels F3 and A2 of Fig. 4 (and Table 2), one can appreciate the convergence in the results independently of whether an experimental strategy based on improved primers or a modelling strategy based on better theoretical estimates of *V* is pursued. After about 60 to 90 min, the slopes of the curves representing $R_a$ and $R_d$ are similar whether the estimates were based on data resulting from fixed or adjusted primers (Fig. 4, panels F3 and A2 respectively). In addition they are similar to those seen in the studies where no primer was used (Fig. 2). Considering that the two studies were different, the values of $R_d$ are identical. The major difference remaining is that the estimate of $R_a$ is higher and the difference, $R_d$–$R_a$, is lower, when one compares the calculation based on the adjusted primer (Fig. 4, A2) to that based on the fixed primer. Both the similarities and the differences can be understood by re-examining equation (15).

We have already pointed out that the second term of this equation contributes substantively to the final estimate of $R_a$. Two aspects of equation (15) need emphasis here: (i) the second term depends on *V* and (ii) the two terms do not change in parallel. This means that the value of *V* will contribute considerably to the determination of the time course of this incongruity and therefore, in a 'net' way, to the profile of $R_a$. As already pointed out, the experimental strategy of adjusted primers, minimises the dependence of the estimate of $R_a$ on the second term and therefore on *V*. The modelling strategy, on the other hand, attempts to obtain the optimal estimate of this volume. The two approaches would therefore be expected to achieve convergent results. They do, but not quite. The reason is that, although the contribution of the second term of equation (15) has decreased with the adjusted primer, it is not zero. The fact that *V* and $pV_D$ are not the same, will continue to exert an influence, as outlined below.

Why are the $R_d$ identical by the two approaches? It can be seen that, in the case of adjusted primers, the dependence of $R_d$ on *V* disappears. This is evident from:

$$R_d = R_a - V\frac{dC}{dt}$$

*which from* (15) *implies*

$$R_d = \frac{C}{C^*}\cdot R^*_a \quad when \quad \frac{dC^*}{dt}\approx 0 \tag{18}$$

The tracer slope is near zero since rendering $C^*$ constant as quickly as possible, is the purpose of the adjusted primer. In one case (fixed primer) the estimated *V* is optimal for the calculation, and in the other (adjusted primer), it is eliminated from the calculation. It is not surprising then, that the estimates of $R_d$ using the two strategies are equivalent.

Why are the $R_a$ calculated by the two approaches still somewhat different? The answer can be surmised by noting that the difference, $R_d$–$R_a$ is proportional to $V$, or:

$$R_a = R_d + V \cdot \frac{dC}{dt} \tag{19}$$

As indicated above, the $R_d$ calculated by the two methods, are the same. The slope of glucose concentrations is not zero. Differences in the estimates of $R_a$ will therefore be based entirely on corresponding differences in the volume, $V$ (multiplied by the slope, often near constant, $dC/dt$). If, as is most often the case, the glucose slope is negative, volumes that are underestimated will lead to $R_a$ that are overestimated (Fig. 4, panel F2 or A2). From an alternative perspective one could also say that $R_d$–$R_a$ is smaller (Fig. 4, panel A2) since the assumed volume that must be depleted of glucose is smaller.

The estimate, $pV_D$, of the volume of the distribution is not however, intrinsic to the method based on adjusted primers. If consistency is to be maintained, the application of $V$, now estimated from the data obtained for the adjusted primer for each subject, should resolve all the differences between the estimates of $R_a$, using the fixed and adjusted primers. Complete convergence between the methods does indeed occur (within the limits of experimental error and the fact that these are two different sets of studies) when the same model is used, as can be seen by comparing panels F3 to A3 (Fig. 4, Table 2). This re-emphasises the concept that the mode of tracer administration is irrelevant when the most appropriate model is used.

Here, we are considering glucose concentrations and fluxes that are changing slowly in time. The effect of the volume of distribution on one-compartment calculations has been considered in the more general non-steady-state system where these fluxes are changing rapidly [63, 93, 94, 95, 96, 97]. Consistently with the present observations, the volume of distribution plays an important part in the final estimate of $R_a$. In highly dynamic situations, it has been suggested that not only the disposal but also the distribution of glucose changes [93, 97].

One anomaly with the present data remains, however, panels F3 and A2 of Fig. 4 show that during the first hour of the study, estimates of $R_a$ (and $R_d$) appear artefactually high. This is equally true for the control subjects (Fig. 4, C3) where this is obviously unlikely. The effects of model order were investigated to gain an understanding of this phenomenon.

*The modelling strategy: effect of model order*

In the first 1 to 2h of the above primed studies, glucose production seems to start at high values and decrease rapidly, followed by a phase where a slower decay in this rate occurs. This applies equally to those studies where fixed, and those where adjusted primers, were used (Fig. 4 A3, C3, F3 and Table 2) when equivalent calculations were made. It is different from the calculations with unprimed tracer infusion (Fig. 2), where this rapid early phase does not seem to occur. What might cause this apparent early phase of increased glucose production? It is, in fact, apparent and it arises because the primer is an injection.

Let us first make a general observation about the model order (or complexity) that is appropriate for a particular analysis. The non-uniformity of glucose distribution in the body is a physical reality that mandates the implementation of models to describe the system [98, 99]. These can be highly complex [100] or, as above, very simple. The complexity of the model structure will be determined by the speed with which changes in metabolic processes or fluxes (read: *EGP*) take place, relative to that of mixing (of newly formed glucose, or exogenously administered tracer). If the latter is rapid in a relative sense, then the system can be considered to be well-mixed. This is undoubtedly why the one-compartment model has worked so well when assessing the responses of the liver, for example, to moderate exercise, meals etc [2, 101, 102, 103, 104, 105, 106, 107]. If, on the other hand, mixing and metabolism take place with similar time-constants, they will necessarily interact and therefore need to be taken into account simultaneously. This necessitates a more complex description of system structure.

This is the anomaly that is introduced by a primed tracer infusion. Although glucose concentrations change slowly, those of the tracer change rapidly following its injection. An injection is an instantaneous (or delta function) input and is necessarily much faster than the distribution processes. The response to this injection, by the discussion above, must therefore be analysed using higher order models.

This was done here by using a two-compartment model. The tracer data were fitted to the solution to equations (A7, A8), namely the function:

$$C_1^* = A_1 \left( I^* - \frac{R_a^*}{b_1} \right) e^{-b_1 t} + A_2 \left( I^* - \frac{R_a^*}{b_2} \right) e^{-b_2 t} \tag{20}$$

The averaged fits are shown (Fig. 5) and account for the early decay of the tracer concentration which is based on mixing. Parameters required in equation (8) were estimated from this fit (Appendix C) and the $R_a$ was calculated from (8). The results are shown in the fourth set of panels in Fig. 4. They continue to be essentially identical for the two primers. Moreover, the early rapid increase in $R_a$ has been eliminated and the pattern for $R_a$ and $R_d$ development during the study now shows the same characteristics as the unprimed tracer infusion of Fig. 2.

The apparent early increased glucose production therefore, arises from the fact that analysis is based on a single compartment model when tracer distribution is changing rapidly following its injection as a primer.

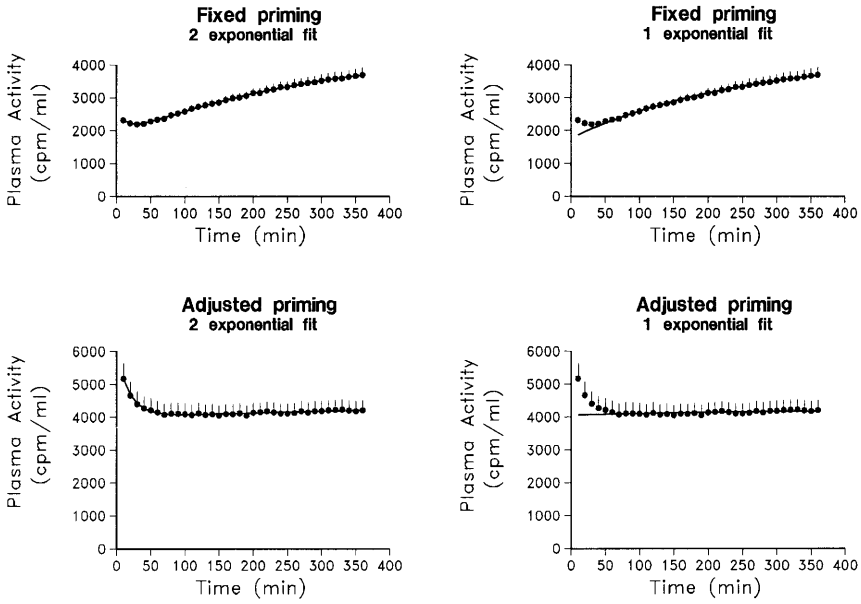



**Fig. 5.** The upper panels show a two-exponential (left panel) and a single-exponential (right panel) fit to the tracer data during tracer infusion in diabetic subjects following a fixed primer. For the single exponential fit, data from the first 50 min were ignored. The lower panels show the same two fits for data obtained in the analogous experiments, but using an adjusted primer

### *Simplifications*

*Model order reduction*

It has already been mentioned that the only reason that a two-compartment model resolves the differences among the calculations is that early tracer data describe the response to its injection. Since the metabolic clearance rate in these diabetic subjects has been shown not to change, a simple stratagem for reducing the number of compartments from two to one is to ignore the first 40 to 50 min of tracer data and use the remaining data to derive estimates of $R_a$ and $R_d$. Figure 5 shows the single exponential fit to the data set with the first 5 points neglected. The resulting single-compartment estimates of $R_a$ and $R_d$ are shown in the fifth set of panels of Fig. 4. They are not different from those in the fourth set respectively, demonstrating the point that the 2-compartment model is only necessary if one wants to account for every tracer data point.

### *Equations based on the adjusted primer and constant tracer concentrations*

The use of an adjusted primer compensates to quite a large extent, for models which may not be perfectly appropriate to the problem in hand. The reason for this was that the slope of the tracer curve, $dC^*/dt$ tended towards zero. Referring once again to equation (15), it is easy to see that, for the adjusted primer, a simplified formula for $R_a$ can be written down:

$$R_a = \frac{R_a^*}{C^*} \cdot C + V \cdot \frac{dC}{dt} = MCR \cdot C + V \cdot \frac{dC}{dt} \qquad (21)$$

$R_a^*$ and $C^*$ are constant. Their ratio therefore corresponds to the constant metabolic clearance rate or *MCR*. *V* (also constant) is the volume of distribution and $dC/dt$ is the slope of the glucose curve. This slope is frequently constant and negative under fasting conditions (Table 1 , Figs. 2, 3). Under conditions where *MCR* and therefore $C^*$ are constant therefore, the expression for $R_a$ corresponds to a monotonically decreasing line of constant slope.

### *Afterthoughts*

*Is the tracer infusion necessary? The tracer injection method*

All the analyses above are predicated on the fact that the metabolic clearance of glucose (*MCR*), or the fractional disappearance rate ($k$) is constant or nearly so. If this was not the case, the data could not be fitted to exponential functions. The adjusted primer (or any primer) would not lead to the constant tracer concentrations found. It is interesting to note that many of the earliest measurements of fasting glucose production in diabetes were based on tracer injections. Exponential fits to tracer data were good and glucose removal, $R_d$, was obtained by multiplying $k$ (obtained from the exponential fit), by the glucose pool size (Table 1). This is compatible with the above developments:

$$\begin{aligned} R_d \quad &= \quad MCR \cdot C \rightarrow R_d = (V \cdot k) \cdot C \rightarrow R_d = k \cdot (V \cdot C) \\ &\rightarrow R_d = k \cdot (glucose\ pool\ size) \end{aligned} \qquad (22)$$

However steady-state was usually assumed and with it, the equality of $R_a$ and $R_d$. Under the non-steady-state conditions which usually prevail in diabetes, equation (19) or (21) are again more accurate expressions.

*The constant specific activity method*

If the sampled specific activity is maintained constant, it can be seen that equations (7) and (8) reduce to (6). To accomplish this in the experimental situation described here, the tracer must be infused at a varying rate, $R_a$*, that would allow $C$* to decrease in parallel to $C$ (Appendix D). This has been the basis of the general non-steady-state method called the 'constant specific activity technique' of measuring $R_a$. Application may be somewhat difficult since a priori knowledge of the rate of decrease of the infusion is not usually available. Nevertheless, minimising changes in $a$ will decrease errors (equation 7) in $R_a$. Maintaining a constant tracer concentration, on the other hand, minimises these errors for $R_d$.

## Summary and discussion

Two important conclusions can be drawn from these analyses. The first is methodological. It is clear that the same physiological picture arises from all tracer protocols used, provided that an analysis appropriate to that protocol is used. Historically this has been done by some investigators. Others have chosen to minimize the necessity of theoretical analysis using experimental strategies. Both approaches are valid, as long as the assumptions and their implications are evaluated.

This was illustrated by the divergence between methods based on 'fixed primers' and 'adjusted primers'. We have shown that these differences arose because the volume of distribution was assigned, rather than estimated from the data. The error arising from this structural assumption applied in both cases, but was minimized when the tracer concentration rapidly approached constancy by optimizing the primer (adjusted primer). Here we have shown that all methods converge, when a consistent analysis was used. Although not a direct validation of the methodology, this consistency among results provides some degree of confidence that physiological estimates of the glucose fluxes are being obtained.

From a physiological perspective, there is a slow decrease in glucose production under overnight fasting conditions from rates that are 20 to 50% higher than normal (depending on the subject population and experimental conditions) to rates that are not distinguishable from the control subjects. It seems that when these rates have stabilised (~constant), they are in the same range as those in healthy control subjects. The fractional disappearance rate (or the metabolic clearance rate) are below normal and remain so throughout the course of observation. Combined with a higher glucose concentration this nevertheless yields glucose utilisation rates which parallel production but are higher. The difference between the two is what drives the decrease in glucose concentrations. Once the concentrations have levelled out, utilisation is equivalent to production and is also normal.

### *Implications of revised analysis in Type II diabetes*

*Increased but decreasing basal rates of* EGP

The recent work described above [23] demonstrated, perhaps somewhat surprisingly, that *MCR* was constant or near constant throughout the (long) day of the basal measurement. Systemic glucose uptake ($R_d$) was therefore directly proportional to the plasma glucose concentration, $C$. As shown by equation (18), glucose production is lower than $R_d$ by a factor which is the product of the volume of distribution and the slope of the glucose curve. Glucose production therefore tended towards a value that at the end of the study was near the values in the matched non-diabetic control subjects. There were clear correlations between glycaemia and *MCR* (negative) and between glycaemia and $R_a$ (positive), at the beginning of the study (overnight fast). The correlation between glucose and *MCR* persisted throughout the day (almost a 24-h fast). The relation between glucose and $R_a$ was, however, completely dissipated by the end of the study when $R_a$ had returned to near normal values [23]. Re-analysis of the data in [22] also showed correlations between $R_a$ and plasma glucose concentrations at the beginning of the day, although these were weaker. They were also no longer present at the end of the 6 h study. The metabolic clearance was lower and again highly correlated to glycaemia at all times.

The morning increase in *EGP*, although it is not high, can vary with the patient population. Thus the increase in a group of newly-diagnosed, diet-treated patients [23] was near 40%, whereas that in another group of patients with established diabetes, was approximately 20%, in spite of higher levels of glycaemia [22]. The contribution of *EGP* to hyperglycaemia is clearly modulated by the MCR. As MCR decreases with the severity of diabetes, ever-decreasing increments in *EGP* are needed for any specific increment in blood glucose (see also equation 5). At the same time, the increasing basal glycaemia could suppress *EGP* [48, 108, 109, 110, 111, 112, 113] to a greater extent.

Changes in *MCR* based on insulin-mediated feedback are fundamental homeostatic mechanisms in metabolism [114]. The fact that *MCR* remains constant (and low) in Type II diabetes, irrespective of glucose

concentrations is likely an expression of the impairments in homeostasis. This suggests that low glucose clearance, which does not respond normally to metabolic stimuli, remains a fundamental lesion in the pathophysiology of Type II diabetes.

Although the increases in $R_a$ are transient, they do seem to account for an important part of the hyperglycaemia seen at certain times of the day, particularly in early diabetes. The cause of these increases has not been completely clarified. Two potential sources for the rises in $R_a$ are the response to a prior meal and an underlying circadian rhythm.

*Effects of prior meal.* Many studies have indicated that during a meal (or insulin) hepatic glucose production in Type II diabetes, is not suppressed to the same extent as that in healthy control subjects [2, 10, 115, 116, 117, 118, 119, 120] and it has been suggested that this lack of suppression is the major contributor to postprandial hyperglycaemia [11]. In this context, Zucker diabetic fatty (ZDF) rats showed a high degree of hepatic insulin resistance and this resistance was relieved by lowering hyperglycaemia using an oral sodium glucose transporter inhibitor [121]. Thus the hyperglycaemia itself might contribute to the increased glucose production. It has been suggested that hepatic glucosamine (arising secondary to hyperglycaemia) could worsen the hyperglycaemia by inhibiting glucokinase and liver glucose uptake, thus interfering with the glucose component of the signal for the suppression of glucose production [122]. The increased basal *EGP* could then itself be partly an expression of hepatic insulin (or glucose) resistance in Type II diabetes. This construct is consistent with the observations in normal dogs [123] that fasting insulin concentrations correspond approximately to the $ED_{50}$ for the action of insulin in suppressing glucose production (starting from zero insulin). It would be expected that the $ED_{50}$, and therefore the operating point, would be higher in an insulin resistant state. This might be true as suggested by the fact that in humans [124, 125] the dose-response curve for the action of insulin on *EGP* parallels that in dogs (over the measurable range in humans) and is shifted to the right in Type II diabetes [80]. Therefore the accentuated hyperglycaemia that follows meals could prolong a period of increased hepatic insulin resistance to basal insulin concentrations and therefore contribute to the increased basal *EGP* and glycaemia. This potential positive feedback loop would therefore, resemble a number of others that characterize Type II diabetes.

Other hormones such as glucagon and the gut hormones are also involved in the response to a mixed meal. In this context, it is interesting to note that GIP has been shown to stimulate glucagon secretion in Type II diabetes [126, 127]. The GIP response could be extended in time, particularly in patients with autonomic neuropathy because of decreases in gastric emptying. Finally, lipotoxic [128, 129] effects mediated through elevated NEFA could contribute to glucose overproduction [130, 131, 132, 133, 134]. All these factors could potentially conspire to increase *EGP* above basal conditions.

*Effects of circadian rhythms.* On the other hand, a meal-independent circadian rhythm has also been suggested to explain the increase in morning EGP. Diurnal variations in the concentrations of glucose as well as in those of triglyceride, urea nitrogen, and cortisol were shown in diabetic but not in control subjects [135]. A similar cycle was then described in glucose tolerance [136] and hepatic glucose production and insulin [71]. Plasma glucose concentrations were highest in the morning (~6:00 to 8:00 h) and fell to their nadir by 16:00 h [71]. This is very similar to the daytime patterns of glycaemia observed in diabetes in [22, 23]. The cyclic changes in EGP have also been shown to occur without any attenuation during a 72-h fast and isoglycaemic clamp in Type II diabetic subjects [137]. They were completely accounted for by changes in glucose infusion rates and were correlated to NEFA and cortisol concentrations [137]. The changes in EGP were, however, not related to glucose (constant) or insulin concentrations, exercise, meals or sleep [137]. These rhythms could be related to the dawn phenomenon which has been shown to occur frequently in both Type I and Type II diabetes [138]. In fact, it seems that cortisol [139] contributes to the dawn phenomenon in patients with Type II diabetes since deletion of cortisol secretion during the night with metyrapone, also decreased the early morning glucose excursion [140]. The correlation between EGP and cortisol [137] is also consistent with a 4 to 6 h delay in its action on glucose metabolism [141, 142, 143]. Other studies have shown an influence of sleep on subsequent glucose metabolism [144, 145]. A nocturnal rise in growth hormone could be implicated by way of its actions on lipolysis from specific adipose depots [146]. At the same time, there is some evidence that basal glycaemia could be controlled centrally at the suprachiasmatic nucleus (SCN) since SCN-lesioned rats lost the 24-h rhythm in basal glucose concentrations [147].

Whether, the cause of the morning increase in EGP in Type II diabetes is related to defective regulation of postabsorptive glucose production or to an accentuated circadian rhythm (dawn phenomenon), it is qualitatively consistent with many of the measurements that have been made. The increase of EGP does not appear to be in the fold-range, as has sometimes been reported but does nevertheless contribute substantively to the hyperglycaemia at this time. The precise nature of the changes will help in the evaluation of the pathophysiologic contribution of EGP to the hyperglycae-

mia of Type II diabetes as well as aid in designing optimal therapies.

### *Implications for $R_d$ or glucose utilisation*

Since glucose concentrations often fall during the first part of the day, it is clear that $R_d$ must be greater than $R_a$. If one makes the further observation that for the non-diabetic subject the two fluxes are equal, then $R_d$ is increased more, relative to the control subject, than $R_a$. In the studies discussed above this increase is over 50%. This is important because this greater influx of glucose into cells is considered to contribute to the peripheral component of glucose toxicity by increasing the flux in the hexosamine pathway [148, 149, 150, 151]. At the same time, the argument that glycaemia increases as a compensation for the decrease in the MCR of glucose (equation 16) to maintain sufficient cellular glucose uptake can still hold, since both $R_a$ and $R_d$ tend towards normal values as the day progresses and thus might help explain the continued increase of glucose concentrations as glucose production 'normalizes'.

Glucose toxic effects could also explain the decreasing *MCR* which seems to occur with disease duration, as was seen in the two groups analysed here. The greater elevations in *EGP* early in the disease also suggest a potentially important contribution of the liver to the development of this toxicity.

### *The importance of the volume of distribution*

We have seen that the use of individually estimated volumes of distribution completely reconciled the calculations of EGP using different tracer protocols (fixed and adjusted primers). This indicates the importance of this parameter in the calculation of EGP. It is also notable that in the three calculations discussed in detail, the volumes of distribution averaged 22% to 25% of body weight for the calculations based on [22] and 18% for those based on [23]. This is from 40% to 90% higher than the traditional estimates which is often near 13% (0.65×20%) ([22, 63], Table 1). The different volumes of distribution in the two analysed studies probably correspond to the different groups of subjects: obese or non-obese, with relatively early or more established diabetes, and indicate that there is some risk in any assumed, rather than calculated, volume of distribution.

It is of interest to consider the meaning of the volume of distribution of glucose in the context of a single-compartment model of glucose kinetics. In a completely well-mixed system, the volume of distribution is simply the physical volume of the space in which the solute is distributed. It has been widely acknowledged [62, 91, 152] however, that this distribution is not uniform. It is considered to be a potentially important problem under highly non-steady-state conditions and has led to a number of efforts to model the non-uniformity in the distribution of glucose, tracer and specific activity using multi-compartment models [93, 94, 95]. Under basal conditions, the relative distribution of glucose and tracer in different compartments is much more likely to remain stable – hence the feasibility of a one-compartment system. Glucose is removed at the cell membrane and it would therefore be anticipated that, under basal conditions there will be a gradient of glucose concentrations between the circulation and the cell membrane or the interstitum [153]. Consistent with this, a recent study showed a lower interstitial glucose concentration both during an IVGTT and under basal conditions in Type I diabetes [154]. A hyperglycaemic hyperinsulinaemic clamp study, however, failed to show such a gradient [155].

It would also be expected that as the fractional disappearance of glucose (*k*, which corresponds to the efficiency of cellular uptake of glucose) increases, such a glucose gradient would also increase so that average (across the extracellular space) concentrations would decrease. The circulating glucose concentration would therefore be ever less representative of this average, implying a lower apparent (or 'effective') volume of distribution. In the single compartment model it was assumed therefore that the effective volume could only be a fraction of the extracellular space. This was called the pool fraction. As we have seen this volume is usually too small. However, when *V* was individually estimated from the data for each subject, a different expression of the hypothesized relation between *V* and *k* was found in a group of subjects with Type II diabetes and their matched control subjects [23]: *V* was strongly and inversely correlated with *k* ($r$=–0.73, $p$=0.0014). Alterations in the glucose volume of distribution are also generally consistent with effects of higher insulin concentrations on this parameter which can increase if glucose phosphorylation becomes rate-limiting, and back-flux of intracellular glucose into the circulation takes place [93, 156]. This does not apply under basal conditions in Type II diabetes [45, 156], or under stimulated conditions if impaired glucose transport is rate-controlling [155].

These suggestions indicate why estimates of the volume of distribution could be much closer to the extracellular fluid volume, or even extend beyond it, as has been seen in the currently discussed studies and those where *V* was calculated from responses to tracer injections [157] (Table 1).

## *Considerations in the measurement of basal glucose production and metabolic clearance: protocol for measuring fasting $R_a$ in Type II diabetes*

We would like to conclude by summarising the different protocols that could be used in evaluating glucose production in diabetes. The intent of this review is to demonstrate that *EGP* could be estimated reliably us-

ing a number of experimental and analytical approaches. The administration of tracer is simply a strategy to 'linearise' the system and obtain an estimate of the metabolic clearance of glucose from plasma. The application of this measure of *MCR* to the circulating concentration of glucose, then yields its production rate. Table 3a, 3b, 3c, summarizes the different possibilities that are used for tracer administration and the formulas that follow on from the different protocols. In their essence, the suggested calculations (i) preclude the assumption of a steady-state (ii) estimate the volume of distribution, *V*, for each study and (iii) often simplify the formula if *MCR* is constant. Table 3a, 3b, 3c, also tries to anticipate some of the problems that can arise if inappropriate assumptions are made. Finally, Appendix D considers the quantitative implications of some assumptions which could be implicit in a number of the analyses which have been used.

It appears that $R_a$ can frequently be related to the plasma glucose concentration, *C*, by a linear relationship: $R_a$ is proportional to *C*, but is translated downward by a constant number, which is the product of the volume of distribution and the slope of the decline in glucose concentration.

**Table 3a.** Summary of formulae for different experimental circumstances and problems that could be encountered

| Experimental Procedure | Calculations | Comments and Potential Problems |
|---|---|---|
| *Tracer injection*<br>Tracer Injection of mass, $I^*$, at $t$=0;<br>Monitor conc of glucose, $C$, and of tracer, $C^*$, over 4h (min 10 points) | Evaluate experimental conditions:<br>(i) $C(t)$ is constant (slope of regression line not significantly different from zero) and $C^*(t)$ fitted well by an exponential function or sum of exponential functions: *Steady state*<br>(ii) $C(t)$ is not constant; $C^*(t)$ fitted well by an exponential function or sum of exponential functions: *Nonsteady state with a constant MCR.*<br>(iii) $C^*(t)$ is not well fitted by exponentials: *Nonsteady state with a time-varying MCR* | |
| | *Constant MCR*<br>$MCR = I^* / \int_0^\infty C^* dt$;<br>$\int_0^\infty C^* dt$ *can be calculated from exponential fit*;<br>$R_d = MCR \cdot C$ | Mixing components emphasized because of injection of tracer.<br>Early part (~20 min) of decay curve may be ignored but this will generate approx. 5% error in *MCR* [158].<br>This will be greater as $k$ increases since mixing and removal kinetics become closer. |
| | (i) *Steady state*<br>$R_d = MCR \cdot C$<br>$R_a = R_d$ | Note that steady-state formulas are independent of $V$ |
| | (ii) *Nonsteady state*<br>*Evaluate V: extrapolate latter part of decay curve to y-axis:*<br>*eg* $\log C^* = A - kt$;<br>$A = \log(\frac{I^*}{V})$: *estimate V*<br>$R_d = MCR \cdot C$<br>$R_a = R_d + V \cdot dC/dt$ | If single exponential fit to data used, may evaluate *MCR* as $kV$. Calculation of $V$ not completely precise although this is a good estimate |
| | (iii) *Time-varying MCR; Nonsteady state*<br>*Can use a nonsteady-state multi-compartment approach but not recommended* | |

**Table 3b.**

<table>
<tr><th>Experimental Procedure</th><th>Calculations</th><th>Comments and Potential Problems</th></tr>
<tr><td rowspan="5"><u>Tracer infusion with adjusted primer.</u><br>Tracer infusion at rate, $R_a^*$ from $t=0$ to $t$=180min<br>Tracer Injection of mass, $I^*$, at $t=0$;<br>$I^* = R_a^* \cdot 100 \cdot \frac{[glucose]_{initial}}{5}$<br><br>Monitor glucose ($C$) and tracer ($C^*$) conc. from $t$=90-120 to $t$=180min with at least 5 data points</td><td colspan="2">Choosing the primer:<br>To the extent that $V$ may vary consistently between different groups of patients, deviations in the calculated and ideal primer will occur. Since $k$ is constant but $C$ is changing, it is not completely clear what glucose concentration to use. One could suggest that the concentration, after it has stabilized would be the most useful, particularly considering equations (16) and (17). From a practical perspective, however, the glucose concentration before starting would be used. This likely works so well in the groups reported [22], since in these groups with established diabetes, the major part of the elevation in fasting glucose concentration is actually determined by the fall in $k$ rather than the increase in production.</td></tr>
<tr><td colspan="2">Evaluate experimental conditions:<br>(i) $C(t)$ and $C^*(t)$ are both constant (slope of regression line not significantly different from zero) from e.g. $t$=90 to $t$=180min: Steady state<br>(ii) $C(t)$ is not constant; $C^*(t)$ is constant: Nonsteady state with a constant MCR.<br>(iii) $C^*(t)$ is not constant: go to 'Tracer infusion with fixed primer'</td></tr>
<tr><td><u>Constant MCR</u><br>$MCR = R_a^* / C^*$<br>$R_d = MCR \cdot C$</td><td>This formula is only applicable when $C^*$ is constant. If not enough data points are collected then $C^*$ may appear constant without being so.<br>Note that $I^*$ is the same as the 'fixed primer' when fasting glucose is 5 mmol/l</td></tr>
<tr><td>(i) <u>Steady state</u><br>$R_d = MCR \cdot C$<br>$R_a = R_d$</td><td>Note that $R_a = R_d = MCR \cdot C = R_a^* \cdot C / C^*$.<br>The specific activity, $a = C^*/C$, may appear constant over a relatively short period of time if $C^*$ (arising from slight overpriming) and $C$ are decreasing at nearly the same rate.</td></tr>
<tr><td>(ii) <u>Nonsteady state</u><br>$V = \frac{I^*}{C^*}$<br>$R_d = MCR \cdot C$<br>$R_a = MCR \cdot C + V \cdot \frac{dC}{dt}$</td><td>$V$ is estimated based on the fact that the primer is near-'perfect'. To the extent that it isn't, there will be an error in $V$. It is nevertheless better than physical estimates based on body weight. An improved estimate may be obtained by fitting the entire course of the tracer data (from $t$=0 to $t$=180).<br>Note: averaging several steady-state estimates of $R_a$ ($=R_a^*/a$) in a nonsteady-state situation does not improve the estimate of $R_a$ since second term ($V \cdot dC/dt$) is not taken into account.</td></tr>
</table>

**Table 3c.**

| Experimental Procedure | Calculations | Comments and Potential Problems |
|---|---|---|
| *Tracer infusion with fixed primer.*<br>Tracer infusion at rate, $R_a^*$ from $t=0$ to t=240min<br>Tracer Injection of mass, $I^*$, at $t=0$;<br>$I^* = R_a^* \cdot 100$<br><br>Monitor glucose ($C$) and tracer ($C^*$) conc. from $t$=150 to $t$=240 min with at least 6 data points.<br><br>Optimally monitor entire time course of $C^*$ until end of study.<br><br>Alternately, extend equilibration time to 6h. | Evaluate experimental conditions:<br>(i) $C(t)$ and $C^*(t)$ are both constant (slope of regression line not significantly different from zero) from e.g. $t$=150 to $t$=240 min or:<br>$C(t)$ is constant and $C^*(t)$ fits equation (12) (below): *Steady state*<br>(ii) $C(t)$ is not constant; $C^*(t)$ is constant or fits equation (12): *Nonsteady state with a constant MCR.*<br>(iii) $C^*(t)$ is not constant and does not fit equation (12): *Nonsteady state with a time-varying MCR.* | |
| | *Constant MCR*<br>$MCR = R_a^* / C^*$ (eq'n 5)<br>*or*<br>$MCR = k \cdot V$,<br>*where:*<br>*k and V identified from:*<br>$C^* = \frac{R_a^*}{V \cdot k} + \frac{1}{V}(I^* - \frac{R_a^*}{k})\, e^{-kt}$<br>(*equation*12) | The first formula is only applicable when $C^*$ is constant.<br>The second formula applies when primer is not 'perfect' and $C^*$ continues to change.<br><br>Note: this analysis applies to both the fixed primer and to an adjusted primer which does not work 'perfectly' in an individual subject. It also applies to unprimed tracer infusion ($I^*=0$). |
| | (i) *Steady state*<br>$R_d = MCR \cdot C$<br>$R_a = R_d$ | Note that $R_a=R_d=MCR \cdot C=R_a^* \cdot C/C^*$. The specific activity, $a=C^*/C$, may appear constant over a relatively short period of time if $C^*$ (arising from some overpriming) and $C$ are decreasing at nearly the same rate. |
| | (ii) *Nonsteady state*<br>$V = I^* / C^*$ *if* $C^*$ *constant, and from eq'n* (12) *if not.*<br>$R_d = MCR \cdot C$<br>$R_a = MCR \cdot C + V \cdot \frac{dC}{dt}$ | Note: $C$ frequently falls linearly so that $dC/dt$ is a constant and negative. The expressions for both $R_a$ and $R_d$ then both correspond to lines with negative slope. |
| | (iii) *Time-varying MCR; Nonsteady state*<br>$MCR = \frac{R_a^*}{C^*} - \frac{V}{C^*} \cdot \frac{dC^*}{dt}$<br>$R_a = MCR \cdot C + V \cdot \frac{dC}{dt}$ | This corresponds to the one-comp't or modified Steele's equation:<br>$R_a = \frac{R_a^*}{a} - \frac{V \cdot C}{a} \cdot \frac{da}{dt}$<br>*where* $a = C^*/C$ (eq'n 7)<br>The estimation of $V$ is more problematic. An approach may be a first pass fit of the data (or part of it) to equation (12).<br>If $MCR$ is increasing gradually, $C^*$ will decrease. It is possible that over a relatively short observation period, $a$ will appear constant. However this is *not* a steady-state and eq'n (5) does not apply. |

*Sources.* As background for this review, a search was carried out on Medline (1966–2001) using the keywords 'glucose production' or 'glucose appearance' and 'diabetes mellitus'. Prior to 1966, personal reference materials were used.

*Acknowledgements.* The authors would like to thank Drs O. Hother-Nielsen and H. Beck-Nielsen for the use of their excellent published data as well as for many discussions. This work has been partly supported by a grant from the Canadian Institutes of Health Research.

## Appendix

### Appendix A: Calculation of rates of metabolic clearance and production of glucose under steady-state conditions, using a tracer injection

From equation (4) and the conservation of (tracer) mass, it is clear that:

$$\int_{0^-}^{\infty} \frac{dM^*}{dt} dt = \int_{0^-}^{\infty} (R_a^* - R_d^*) dt \tag{A1}$$

where $M^*$ is the mass of the tracer in the system and $R_a{}^*$ and $R_d{}^*$ are its input and output. $0^-$ is a time just before zero. If the tracer input is an injection, then: $R_a{}^*(t)=I^*\delta(t)$ where $I^*$ is the total amount injected and $\delta(t)$ is a delta function. This also means that $M^*$ is zero both just before the tracer injection ($t=0^-$), and at $\infty$ when the tracer has been cleared from the system so that we have:

$$\begin{aligned} M^*|_{0^-}^{\infty} &= 0 = \int_{0^-}^{\infty} \left(R_a^* - R_d^*\right) dt \\ &\quad or \\ \int_{0^-}^{\infty} R_a^* dt &= \int_{0^-}^{\infty} I(t)^* dt = I^* = \int_{0^-}^{\infty} R_d^* dt \end{aligned} \tag{A2}$$

Let us now define the metabolic clearance rate, *MCR*, in the sense of DiStefano [159, 160], as the net rate of removal of glucose from plasma per unit of concentration in plasma, or as the volume of plasma from which glucose is completely removed per unit of time. Then, if and only if *MCR* is constant, we can say:

$$\begin{aligned} \int_{0^-}^{\infty} R_d^* dt &= \int_{0^-}^{\infty} MCR \cdot C^* dt = MCR \cdot \int_{0^-}^{\infty} C^* dt \\ &\quad and \\ MCR &= \frac{I^*}{\int_{0^-}^{\infty} C^* dt} \end{aligned} \tag{A3}$$

In a compartmental system, the decay after an injection is characterized as the sum of exponentials, $C^* = A_1 e^{-b_1 t} + A_2 e^{-b_2 t} + \ldots$, leading to the expression:

$$MCR = \frac{I^*}{\frac{A_1}{b_1} + \frac{A_2}{b_2} + \ldots} \tag{A4}$$

that is, *MCR* equals the amount of tracer injected divided by the area under its decay curve.

### Appendix B: Modelling inhomogeneity in the glucose system

The non-uniformity of glucose distribution throughout the body is characterized by gradients in tissue interstitial spaces as well as within the circulation itself. Figure 6 illustrates models of different degrees of complexity that have been used to address the problem of this distribution in the context of calculating the rate of endogenous glucose production.

*The distributed system.* The first diagram illustrates the general inhomogeneous system with all parameters varying in both space and time. Sampling and input (glucose production) are however assumed to occur within a well-mixed space. Equations can be written down for such a system [161, 162, 163, 164, 165]. There is no general solution. Within certain constraints (time invariance for all parameters but one, glucose removal from the sampling compartment), a general equation can be written down, describing the relation between the measured concentration, the input (*EGP*, $R_a$) and metabolic clearance [$Vk$ or $V(k_0+\Delta k)$]:

$$\begin{aligned} C_1(t) &= C_1(0) - R_a(0) \cdot \int_0^t h(\tau) d\tau \\ &\quad + \int_0^t h(t-\tau) \cdot [V \Delta k(\tau) C_1(\tau) + R_a(\tau)] dt \end{aligned} \tag{B1}$$

Essentially the same equation holds for the tracer:

$$\begin{aligned} C_1^*(t) &= C_1^*(0) - R_a^*(0) \cdot \int_0^t h(\tau) d\tau \\ &\quad + \int_0^t h(t-\tau) \cdot [V \Delta k(\tau) C_1^*(\tau) + R_a^*(\tau)] d\tau \end{aligned} \tag{B2}$$

where:

$C_1(t)$ = glucose concentration in sampling compartment
$C^*_1(t)$ = tracer concentration in sampling compartment
$R_a(t)$ = glucose production rate
$R^*_a(t)$ = tracer infusion rate
$R_d(t)$ = glucose disappearance (removal) rate
$R_{d1}(t)$ = glucose disappearance rate from sampling compartment
$R_{d1}(t)$ = $V(k_0+\Delta k(t))C_1(t)$
$V_1$ = volume of distribution of sampling compartment
$k$ = fractional disappearance rate of glucose= $k_0$(constant)+$\Delta k(t)$
$h(t)$ = impulse response function (concentration after tracer injection)

Using equations (B1) and (B2), both glucose appearance and disappearance can be extracted by sequential deconvolution [163]. Of note, the relation is expressed in terms of $h(t)$, which is the impulse response function (glucose tracer concentration response to an injection of the tracer) for the time-invariant version of the system.

*The one-compartment model.* The second model in Fig. 6 is the one-compartment model on which most non-steady-state calculations of *EGP* have been made. As is clear, it assumes that glucose distribution throughout the whole body takes place so rapidly that it does not affect the estimation of the process in question: *EGP*. The analysis of this equation can start with equations (3) and (4), by defining $M=VC$ and $M^*=VC^*$, since glucose is assumed to be uniformly distributed at a single concentration, $C$. $V$ is the volume of distribution, equal to the volume of the single compartment.

Using this definition and equations (2), (3) and (4), we can very easily show that the glucose and tracer systems can be described by:

$$\frac{dC}{dt} = -k \cdot C + \frac{R_a}{V} \quad and \quad \frac{dC^*}{dt} = -k \cdot C^* + \frac{R_a^*}{V} \tag{B3}$$

since from (2), $R_d$ is proportional to $C$ and $R_d{}^*$ is proportional to $C^*$ with the same factor describing this proportionality in both cases. This factor is termed the metabolic clearance rate (*MCR*) since it also describes the efficiency of glucose remov-

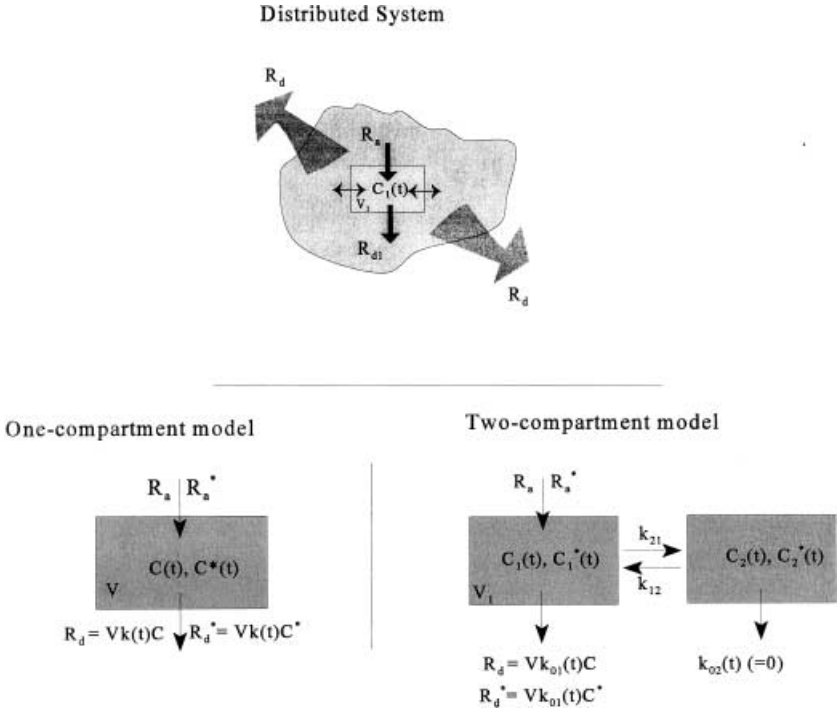



**Fig. 6.** The top panel depicts a general distributed system. Glucose enters in a well-mixed pool which is embedded within this system. It exchanges with the remainder of the system where distribution is characterized by convection, diffusion and transport processes. It is eliminated at the cell surface or within cells throughout the system. The lower panels illustrate models of the system which are used in the calculations in this review. These are the one-compartment model (left panel) and the two-compartment model (right panel). Parameters are defined in the text and the abbreviations

al, i.e. relatively independently of the glucose concentration. It is also equal to $Vk$, where $k$ is the fractional disappearance rate. In general, $k$ is time-dependent. It can be eliminated from equations (B3) to yield the traditional formula for glucose production [62, 91]:

$$R_a = \frac{R_a^*}{a} - \frac{V \cdot C}{a} \cdot \frac{da}{dt} \tag{B4}$$

where: $C(t)$, $C^*(t)$: glucose and tracer concentrations

$R_a(t)$, $R^*_a(t)$: glucose and tracer appearance rates
$V$: volume of distribution
$a$: specific activity or enrichment; $a=C^*(t)/C(t)$

*Two-compartment model.* The third model in Fig. 6 compresses all the inhomogeneity of the system into a single compartment, additional to the input or sampling compartment. The equations can be written as:

$$\frac{dC_1}{dt} = -(k_{21} + k_{01}) \cdot C_1 + k_{12}C_2 + \frac{R_a}{V_1} \tag{B5}$$

$$\frac{dC_2}{dt} = k_{21}C_1 - (k_{12} + k_{02}) \cdot C_2 \tag{B6}$$

where: $k_{12}$ and $k_{21}$: exchange coefficients

$k_{01}$ and $k_{02}$: fractional disappearance rates

Analogous equations hold for the tracer:

$$\frac{dC_1^*}{dt} = -(k_{21} + k_{01}) \cdot C_1^* + k_{12}C_2^* + \frac{R_a^*}{V_1} \tag{B7}$$

$$\frac{dC_2^*}{dt} = k_{21}C_1^* - (k_{12} + k_{02}) \cdot C_2^* \tag{B8}$$

For the special case of the two-compartment model where $k_{02}$=0, there is a closed form solution [94, 160, 163]:

$$R_a(t) = \frac{R_a^*}{a_1} - \frac{V_1 C_1}{a_1} \cdot \frac{da_1}{dt} + V_1 k_{12} k_{21} \int_0^t e^{-k_{12}(t-\tau)} \left[ \frac{C_1^*(\tau)}{a_1(t)} - C_1(\tau) \right] d\tau + terms\ defining\ initial\ conditions \tag{B9}$$

where: $C_1(t)$, $C^*_1(t)$: glucose and tracer concentrations

$R_a(t)$, $R^*_a(t)$: glucose and tracer appearance rates
$V_1$: volume of distribution
$a_1$: specific activity or enrichment in measured compartment; $a_1=C^*_1(t)/C(t)$

In this section we illustrate that solutions at various levels of complexity exist. The application of a specific model is dependent on the problem which is being addressed.

## Appendix C: Parameter identification for a two-compartment model

The equations describing the two compartment system considered here are given by (B5, B6, B7, B8).

A closed form of the solution that illustrates the progressive nature of the 'corrections' as model order increases, is given by (B9). If all parameters are constant, the solution to (B7–B8) for a tracer injection at time zero, $I^*\delta(t)$, and an infusion starting at $t$=0, $R_a^*H(t)$ where $H(t)$ is the Heavyside function, is given by equation (20), where

$$C_1^* = A_1 e^{-b_1 t} + A_2 e^{-b_2 t} \tag{C1}$$

is the response for a unit injection, $\delta(t)$. When the parameters $A_1$, $A_2$, $b_1$ and $b_2$ are estimated from (20), using the tracer data

shown in Fig. 2 and 3, model-specific parameters used in (8) can be calculated from:

$$\begin{aligned} k_{12} &= \frac{b_1A_2+b_2A_1}{A_1+A_2} \\ k_{21} &= \frac{A_1A_2(b_1-b_2)^2}{(A_1+A_2)(b_1A_2+b_2A_1)} \\ k_{01} &= \frac{b_1b_2(A_1+A_2)}{b_1A_2+b_2A_1} \\ V_1 &= \frac{1}{A_1+A_2} \end{aligned} \quad \text{(C2)}$$

### Appendix D: Quantitative evaluation of specific experimental scenarios

Here we briefly consider the implications of some of the assumptions which could be implicit in the calculations of $R_a$ which have been made in Type II diabetes. These will be considered on the background of a decreasing glucose concentration, which could however not always be present. Moreover, we will assume that this concentration will be decreasing in a linear fashion. We therefore have:

$$\frac{dC}{dt} < 0 \quad and \quad \sim constant \quad \text{(D1)}$$

Also recall the following equations which describe the relations between the glucose concentration, $C$, its endogenous production, $R_a$, and its utilisation, $R_d$, within the confines of a one-compartment system of volume, $V$:

$$V \cdot \frac{dC}{dt} = R_a - R_d \quad or \quad R_d = R_a - V \cdot \frac{dC}{dt} \; R_a = \frac{R_a^*}{a} - \frac{V \cdot C}{a} \cdot \frac{da}{dt} \quad \text{(D3)}$$

$$R_a = \frac{C}{C^*} \cdot R_a^* + V \cdot \left[ \frac{dC}{dt} - \frac{C}{C^*} \cdot \frac{dC^*}{dt} \right] \quad \text{(D4)}$$

Let us now examine the following conditions:

*(i) C* is constant, or more generally, MCR is constant.* Under these circumstances, which were already considered, we have, from equations (D4) and (D2):

$$\begin{aligned} R_a &= \frac{R_a^*}{a} + V \cdot \frac{dC}{dt} \\ R_d &= \frac{R_a^*}{a} \end{aligned}$$

*(ii) a is constant.* This implies that $C$ and $C^*$ are changing exactly in parallel. Since, $C$ is decreasing, then so must be $C^*$. This implies an increasing *MCR*. This is consistent with the following:

$$\begin{aligned} R_a &= \frac{R_a^*}{a} \sim constant_1 \\ R_d &= \frac{R_a^*}{a} - V \cdot \frac{dC}{dt} \sim constant_2 \\ MCR &= \frac{constant_2}{C} \end{aligned}$$

*(iii) $R_a$ and MCR are both constant.* This corresponds to the situation which is implied when $C$ is seen to decrease, $R_a$ is found to be normal and *MCR* is constant and reduced [52, 84]. It could arise, for example, after meals where a suppressed (or partially suppressed, or even not suppressed at all) glucose production returns to a normal constant rate. At the same time the immediately post-absorptive glucose concentration is high and decaying slowly because clearance is low. This situation for glucose is analogous to that for an overprimed tracer infusion and could be described, similarly to equation (12), as:

$$C = \frac{R_a}{V \cdot k} + \left( C(0) - \frac{R_a}{Vk} \right) e^{-kt}$$

If $C(0) > R_a/Vk$, then $C$ would decrease exponentially from postprandial concentrations, $C(0)$, to an eventual equilibrium concentration of $R_a/(Vk)$. This would, of course, be high because $k$ is low. This is a simple and attractive solution. It is, however, not compatible with those studies where a linear decrease in glucose is seen many hours after a meal (e.g. [22, 23]).

All eventualities should be considered in data interpretation and the one which is most compatible with a particular data set, chosen in its discussion.

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