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Rapid communication

Production and metabolic clearance of glucose under basal conditions in Type II (non-insulin-dependent) diabetes mellitus

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Abstract

Aims/hypothesis. The pathogenesis of fasting hyperglycaemia in Type II (non-insulin-dependent) diabetes mellitus has yet to be clarified. Rates of glucose production (R_a), utilization and metabolic clearance rate were therefore measured during an extended fast, in control subjects and in Type II diabetic patients.

Methods. Nine subjects with newly-diagnosed or diet-treated diabetes and seven control subjects matched for age and weight (BMI 36.0 ± 2.4 and 35.3 ± 3.1 kg/m^2 respectively) underwent an overnight fast followed by a 10-h unprimed infusion of [6-^{3}H]glucose. Plasma tracer concentrations were fitted by a single-compartment model.

Results. The metabolic clearance rate was near-constant [61.7 + 2.4 ml/(min-m^2)] in diabetic patients and [75.5 ± 3.3 ml/(min-m^2)] in control subjects ($p < 0.05$). It was correlated to the glucose concentrations both at $t = 0$ ($r = –0.752$, $p = 0.0008$) and $t = 10$ h ($r = –0.675$, $p = 0.004$). The calculated volume of distribution was 17.3 ± 1.41 (18.2 % weight, diabetes), 19.6 ± 2.41 (18.4 % weight, control). Glycaemia fell from 10.7 ± 0.8 mmol/l to 6.5 ± 0.3 mmol/l by 10 h ($p < 0.05$) in diabetes and from 5.6 ± 0.6 to 4.8 ± 0.1 mmol/l in control subjects ($p < 0.05$). The rate of glucose production decreased in parallel, from 563 ± 33 to 363 ± 23 μmol/(min-m^2) ($p < 0.05$) in diabetes from 419 ± 20 to 347 ± 32 μmol/(min-m^2) in control subjects. Initial R_a was higher in diabetic patients than in control subjects ($p < 0.05$) and was highly correlated to glycaemia ($r = 0.836$, $p = 0.0001$). By 10 h, R_a had converged in diabetic patients and control subjects and all correlation with glycaemia was lost ($r = 0.0017$, $p = 0.95$).

Conclusions/interpretation. In relatively early diabetes, the more "labile" portion of fasting hyperglycaemia, which subsequently decreased, was closely related to the simultaneously decreasing R_a. The 25 % increase in glucose concentrations which persisted as stabilized R_a, resulted from about a 20 % lower metabolic clearance rate. [Diabetologia (2001) 44: 983–991]

Keywords Glucose production, metabolic clearance of glucose, compartmental analysis, fasting hyperglycaemia.

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Abbreviations: MCR, metabolic clearance rate of glucose; *Ra*, rate of appearance (production) of glucose; *Rd*, rate of disappearance (utilization) of glucose; *Ra**, rate of tracer infusion; *Rd**, rate of tracer disappearance; *I**, tracer injection (primer); *C*, glucose concentration; *C**, tracer concentration; *a*, specific activity (*C**/*C*); *V*, volume of distribution; *K*, fractional disappearance rate of glucose; *t*, time

The relative importance of glucose over-production and inadequate peripheral removal in the postabsorptive (fasting) hyperglycaemia of Type II (non-insulin dependent) diabetes mellitus has produced diverse estimates. Some studies show that a strong correlation exists between fasting glucose concentrations and its production over a wide range of glycaemia, suggesting a causative role for excessive glucose output in hyperglycaemia [1, 8]. Other measurements, however, show almost no correlation between glucose production rates and concentrations [9–14],

particularly lower glucose concentrations [15], although at all concentrations of glucose (and insulin) which prevail in diabetes, these rates are clearly excessive. Other studies show an attenuated association with small increases in glucose output as glycaemia increases [16–19]. A correlation is nevertheless maintained. Inevitably, the importance of the role of impairments in the metabolic clearance rate of glucose varies inversely with that of its production. The extent to which this is physiological or methodological is not clear.

The answers to this question can contribute to a better understanding of the pathophysiology of diabetes and direct the search for treatment targets. In all cases, the measurements of glucose fluxes were made using tracer methods: an infusion of labelled glucose after a priming injection. Glucose production was calculated either as the ratio of the tracer infusion rate and the plasma specific activity or enrichment of glucose, if the measurements of the latter were deemed to be constant or, using non-steady state formulas, primarily based on a single compartment model of glucose kinetics [20,21]. This study therefore re-examined the tracer-based calculation of glucose production in diabetes and the implication of the assumptions made on the calculated rates.

Subjects and methods

Sixteen subjects participated in these studies. Nine had newly-diagnosed or diet-treated Type II diabetes and seven were control subjects matched for age and weight with normal glucose tolerance. Table 1 summarizes the subject phenotypes. The studies were approved by the human ethics committee of the Ottawa Hospital and all participants gave their informed consent. After an overnight (12–14 h) fast, an unprimed infusion of [6-^{3}H]glucose was initiated and continued for 10 h (except for two subjects where it was terminated after 8 h). Samples were collected for glucose and tracer measurements on a variable schedule related to the anticipated rapidity of changes in tracer concentrations but at least every 20 min.

Materials. [6-^{3}H]glucose was obtained from Amersham Corporation (Arlington Heights, Ill., USA) and was purified using ion-exchange HPLC (column: HPX-87P, Biorad, Hercules Calif., USA) and tested for sterility and pyrogenicity.

Analytical methods. All samples for tracer measurements were prepared by deproteinization [22]. The supernatant was passed through an ion-exchange resin (Dowex 1-X8, Biorad, Richmond,Calif., USA) to remove acid components. An aliquot of the eluate was then evaporated at 60° C, the residue redissolved in water and Formula 989 (NEN, Boston,Mass.,USA) and the radioactivity determined in a liquid scintillation counter (Tricarb 2200CA, Canberra Packard, Dowers Grove, Ill., USA). Radioactivity in the sixth position was further verified by dimedone-precipitation of the formaldehyde after the periodate oxidation of glucose [22].

Calculations. All tracer data in each study was fitted to a function representing integrated exponentials using the method of steepest descent and the Marquardt algorithm [23]. One- and two-exponential fits were compared using the chi-square test. In all cases, a single exponential was sufficient and therefore a one-compartment approach was used to calculate the appearance and metabolic clearance rates of glucose. Goodness of fit was expressed as the sum of squares of fractional residuals and a runs test was done to assess the random nature of the residuals. The goodness of fit was visually confirmed by examining the residuals (observed minus fitted values) using a cumulative frequency plot [24]. Ordered residual values were plotted against the 'Z-value', characteristic of normally distributed residuals (Gaussian distribution). The Z-value corresponds to the number of estimated standard deviations from the mean (which is zero for residuals).

The glucose and tracer kinetics are described by:

$$\frac{dC}{dt} = \frac{R_a}{V} - k \cdot C \quad (1)$$

$$\frac{dC^*}{dt} = \frac{R_a^*}{V} - k \cdot C^* \quad (2)$$

where C and C^* are the concentrations of glucose and tracer respectively. R_a and R_a^* are the rates of appearance of glucose and infusion of tracer. k is the fractional rate of glucose disappearance and V is the volume of distribution. On the assumption that k is constant, the solution to equation (2) can be expressed as:

$$C^* = \frac{A}{k}(1 - e^{-kt}) \text{ where } A = \frac{R_a^*}{V} \quad (3)$$

When A and k are identified, V can be estimated from (3). With (a constant) k and V known, R_a can be calculated from (1), using the measured values of glucose, C. It should be noted that the method used here is equivalent to the general approach described previously [20, 21], with basal tracer concentrations, C^*, described by the function in (3), although an independent estimate of V was obtained in this case.

Although the conditions of this study are basal, some variation in k might nevertheless be expected. When the runs test indicated that the residuals were not completely random, deviations in the fractional disappearance rate were allowed so that it varied as $k + \Delta k$ where Δk is a function of time and k was determined from the one-compartment fit. This would account for variations in tracer concentration, ΔC^* from the solution, C^*, described in (3). If both ΔC^* and Δk are assumed small, they would then be related by the equation:

$$\frac{d\Delta C^*}{dt} = -k\Delta C^* - C^*\Delta k \quad (4)$$

Equation (4) can be solved for $\Delta k(t)$ and provides estimates of deviations in k from the constant value.

After the determination of k from equation (2), R_a was determined from equation (1). The rate of glucose disappearance or utilization, R_d, was then calculated as:

$$R_d = V \cdot k \cdot C \text{ or } R_d = V \cdot (k + \Delta k) \cdot C \quad (5)$$

Comparisons between early and late glucose production rates and metabolic clearance in diabetic patients or control subjects, were done using two-way analysis of variance. Regression analysis was used and correlation coefficients calculated in relating R_a and R_d to the ambient glycaemia. SAS (SAS Institute, Cary, N.C., USA) software was used. Results are presented as means ± standard errors of the mean and rates are calculated per m^2 of body surface area.

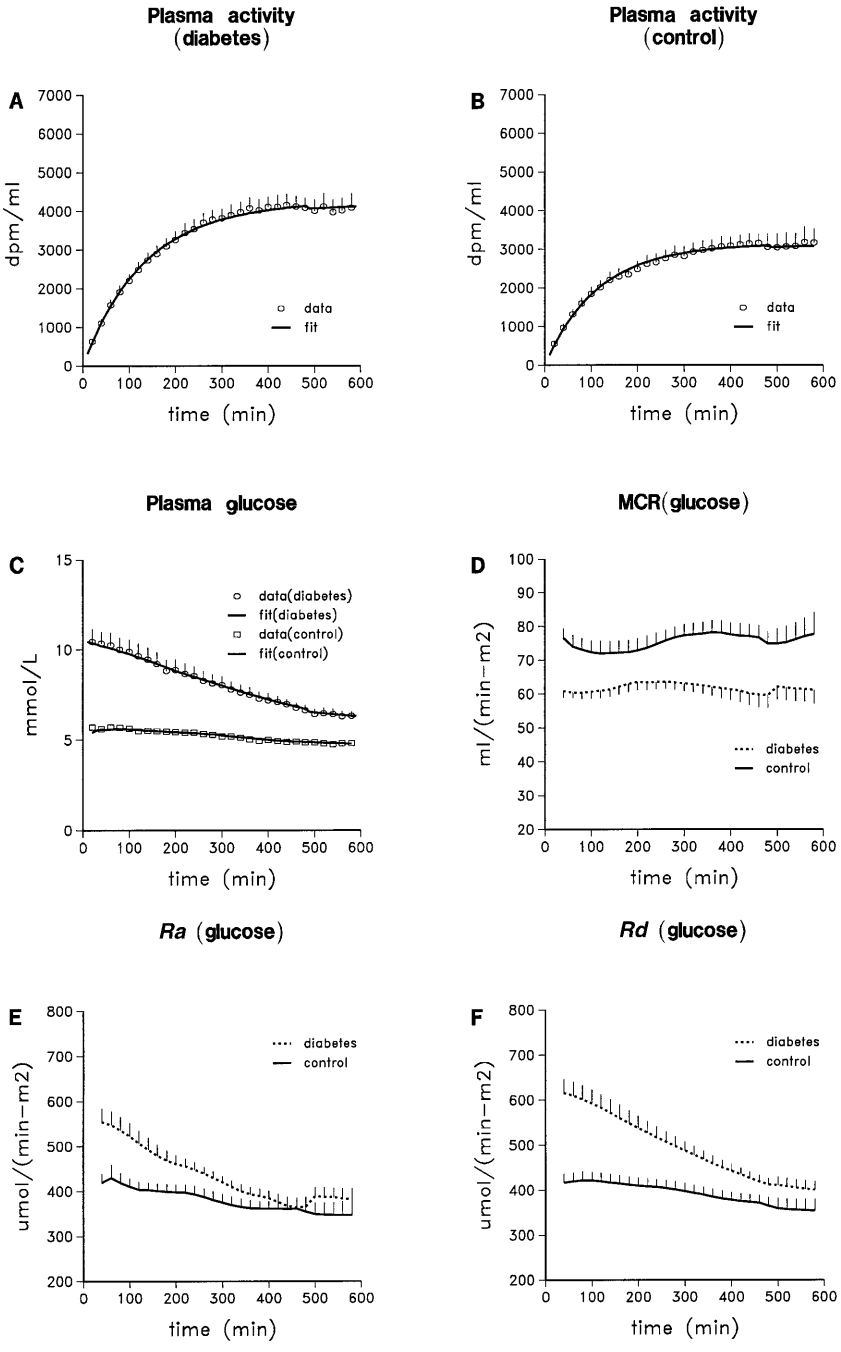

Fig. 1 A–F. Summary of results. **A** Plasma tracer concentrations during a 10 h infusion of [6-^{3}H]glucose in subjects with Type II diabetes. The solid line represents the best fit to equation (2) or (3). **B** The analogous tracer concentrations and fit in control subjects. **C** Plasma glucose concentrations throughout the 10 h experimental period in patients with Type II diabetes and non-diabetic control subjects. **D** The metabolic clearance rate (*MCR*) of glucose in diabetic patients and control subjects. **E** Glucose production rates (R_a) in Type II diabetic patients and in control subjects. **F** Glucose utilization rates in diabetic patients and control subjects

Results

Glycaemia remains increased throughout in diabetes ($p < 0.05$) and decreases over the course of the study – from 10.7 ± 0.8 to 6.5 ± 0.2 mmol/l ($p < 0.05$) in diabetic patients and from 5.6 ± 0.06 to 4.8 ± 0.1 mmol/l in control subjects ($p < 0.05$) (Fig. 1C). The decline is nearly linear with a slope of 7.2 ± 1.2 μmol/l per min in diabetic patients and 1.3 μmol/l in control subjects throughout most of the study. Concentrations of [6-^{3}H]glucose and averages of the fits to the integrated single exponential function (or, equivalently, the one-compartment model are shown in Figure 1A, B.). The sum of squares of fractional residuals was al-

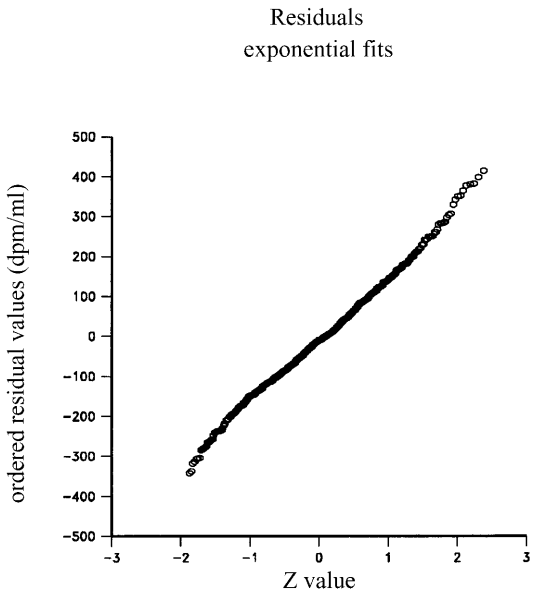

Fig. 2. Cumulative frequency plots for the residuals resulting from the single-compartment fits to the tracer data. Ordered residual values are plotted against the Z-value which represents the number of estimated standard deviations from the mean value (= 0) and is representative of normally distributed residuals. The plot is nearly linear showing randomly distributed residuals

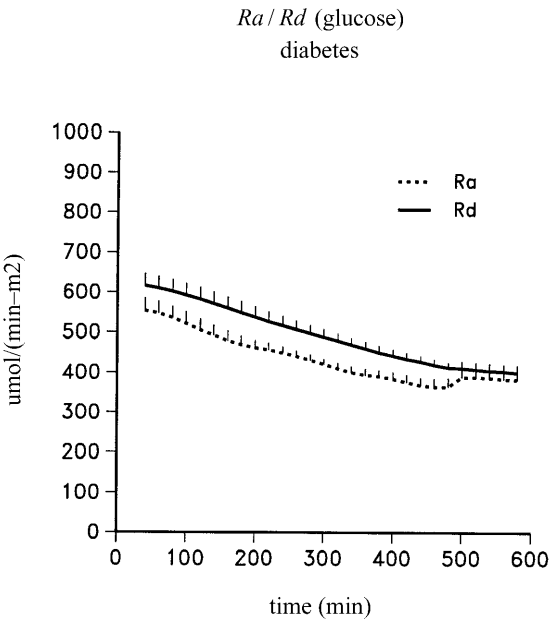

Fig. 3. Rates of glucose production (R_a, . . .) and utilization (Rd, —) are simultaneously plotted for the diabetic patients to demonstrate the approximate constant difference between them which drives the decline in the glucose concentrations

ways less than 0.005, indicating good conformity of the data to the model. A visual demonstration of the sufficiency of the model explaining the time-course of the tracer data, was obtained by plotting the residuals against Z-values (number of estimated standard deviations from mean residual value of zero) which would characterize residuals with a Gaussian distribution (Fig. 2). The fact that the points (from all fits) closely follow a line with slope corresponding to the standard deviation, strongly suggests that, the single compartment model is sufficient to describe these data. Runs tests also indicated that in most (all but two) cases, the residuals were randomly distributed.

When this was not the case, $\Delta k(t)$ was estimated using equation (4) and MCR as a product of $k + \Delta k$ and the volume of distribution, V. The calculated MCR, when variations in k were allowed is shown in Figure 1D. Metabolic clearance rates remained nearly constant, corroborating the assumptions made on a constant value for this parameter. The MCR was 61.7 ± 2.4 ml $\cdot$ min$^{-1}\cdot$m^{-2} in diabetic patients and 75.5 ± 3.3 ml$\cdot$min$^{-1}\cdot$m^{-2} in control subjects respectively ($p < 0.05$). The volume of distribution, V, calculated from equation [3], was 17.3 ± 1.4 l and 19.6 ± 2.3 l in diabetic patients and control subjects respectively. This corresponded to 18.2 % and 18.4 % of the body weights and was not significantly different.

The calculated rates of glucose production R_a, in the two groups are in Figure 1E. In the diabetic group, R_a decreased from 563 ± 33 to 363 ± 23 μmol $\cdot$ min$^{-1}\cdot$ m^{-2} at $t = 480$ min ($n = 9$) and to 377 ± 22 μmol $\cdot$ min$^{-1}\cdot$ m^{-2} at $t = 600$ ($n = 7$, $p < 0.05$). In the control group, R_a decreased from 419 ± 20 to 347 ± 32 μmol $\cdot$ min$^{-1}\cdot$ m^{-2} ($p < 0.05$). In the diabetic group, R_d declined, parallel with the R_a, from 628 ± 35 to 412 ± 14 ($t = 480$ min, $n = 9$) and then to 399 ± 20 μmol $\cdot$ min$^{-1}\cdot$ m^{-2} ($t = 600$ min, $n = 7$, $p < 0.05$) (Fig. 1F). R_d was also consistently higher than R_a until $t = 480$ min ($p < 0.05$). In the control group, R_d decreased from 427 ± 16 to 373 ± 32 μmol $\cdot$ min$^{-1}\cdot$ m^{-2}. R_a and R_d, however, were not distinguishable throughout the study. The parallel course followed by R_a and R_d in the diabetic patients is shown in Figure 3. The nearly constant difference, $R_d - R_a$, explains the decline in C, which is linear in time.

At $t = 0$, R_a was greater in the diabetic patients than in the control subjects ($p < 0.05$). The difference was lost by the end of the study. Although attenuated, similar comparisons held for the control subjects. This suggests a relation between R_a and glycaemia (Fig 4). Linear regressions and correlation coefficients were assessed at the beginning and end of the study when glucose concentrations were lower. At the beginning of the study, R_a was highly correlated with glucose concentrations. By the end, however, this correlation was completely lost. In contrast MCR remained closely correlated to the glycaemia even as this decreased to 60 % of its starting value.

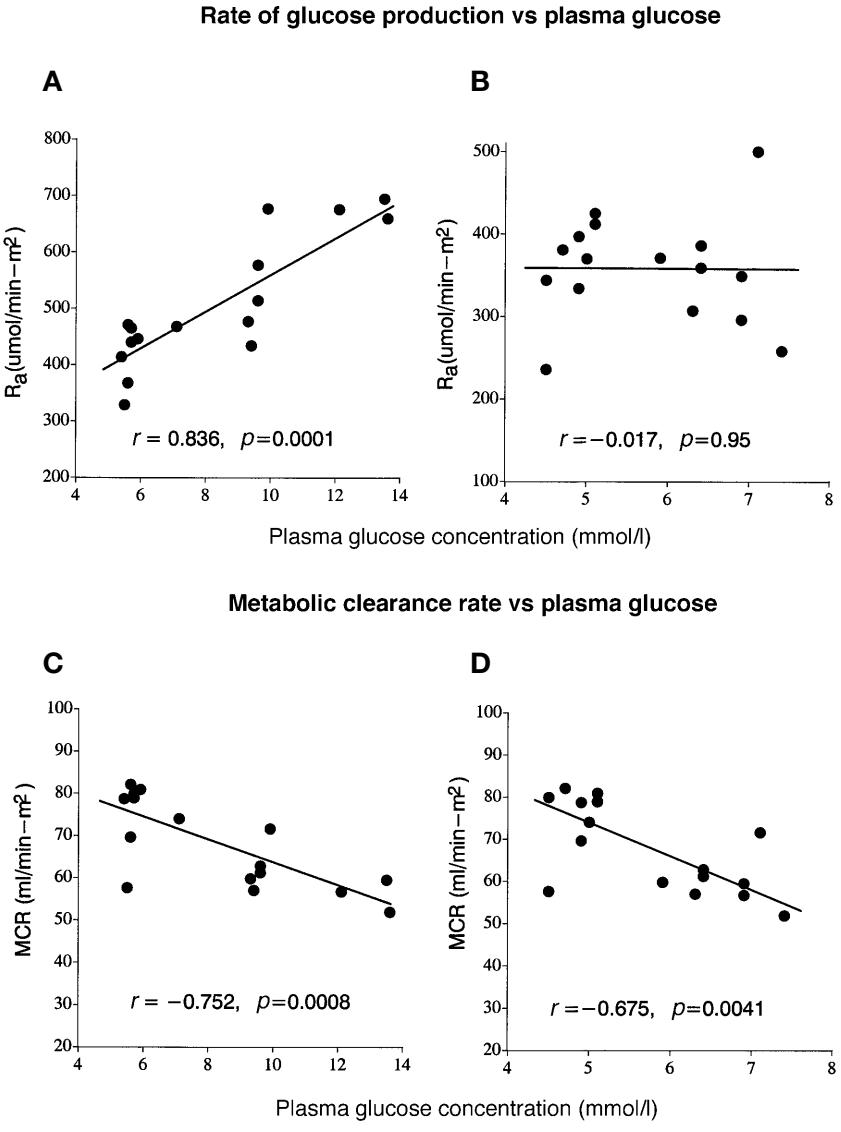

Fig. 4. Correlations between the glucose production rate and glucose concentration in all subjects, early in the study (**A**) (t = 0 min, $r = 0.836$, $p = 0.0001$) and at the termination of the study (**B**) (t = 10 h, $r = -0.017$, $p = 0.95$). Correlations between the metabolic clearance rate (MCR) and glucose concentrations in all subjects at the beginning (**C**) (t = 0 min, $r = -0.752$, $p = 0.0008$) and end (**D**) (t = 10 h, $r = -0.675$, $p = 0.0041$) of the observation period. It can be seen that the early strong correlation between glucose production and concentrations is lost as glycaemia stabilizes in diabetes. The relation between the MCR and glucose concentrations however persists and explains the increase in the late, stabilized glucose concentrations

Discussion

We have shown that glucose production rates in patients with relatively early Type II diabetes, are correlated to its concentrations, but not all the time. They are increased above the rates seen in matched subjects who are normoglycaemic but not all the time. They are however, always related to the metabolic clearance rate, which despite the changing glycaemia, remains near constant during a 10 h fast. The MCR is moreover, somewhat lower in diabetic patients, than in non-diabetic subjects, but not as much as it would be expected if MCR was the only determinant of glycaemia. These results could also reflect the divergent results seen in the many measurements made of these fluxes in diabetes and their relations, causal or otherwise, to glucose concentrations. These disparities could arise from methodological differences of the studies, analytical differences and different patient populations.

In these studies as in many others [1–3, 8–11, 13, 14, 17–19] where sampling takes place over a sufficiently long time, plasma glucose concentrations are seen to continue declining, particularly in Type II diabetes, over several hours after an overnight fast. This is clearly not found in every subject but the assumption must be made in general. It immediately implies

Table 1. Subject characteristics

	n	Sex (men/women)	Age (years)	Weight (kg)	BMI (kg/m^2)	F BG (mmol/l)
Type II diabetes	9	5/4	47±4	106.3±11.2	36.0±2.4	10.7±0.8
Control subjects	7	3/4	41±3	95.2±6.4	35.3±3.1	5.6±0.06

that some error could be incurred in glucose flux calculations in diabetes, if 'basal' is equated with 'steady-state'. In order to gain insight into the sources of error, we can examine the traditional formula for R_a, under nonsteady-state conditions, in a one-compartment system [20, 21]. This is derived by eliminating k from equations (1) and (2):

$$R_a = \frac{R_a^*}{a} - \frac{V \cdot C}{a} \cdot \frac{da}{dt} \text{ or } \frac{1}{a} \cdot (R_a^* - V \cdot C \cdot \frac{da}{dt}) \qquad (6)$$

where p, the 'pool fraction' [20, 21], is incorporated into V, which can be considered the effective volume of distribution. Frequently, and certainly at the start of an unprimed tracer infusion, the slope of the specific activity or enrichment, $a\ (= C^*/C)$, will be positive so that (6) represents the small difference between two larger terms, which increases the potential error (since this is summed for the two terms). Moreover, any errors will be amplified by dividing by a, a small number since at the beginning of the tracer infusion, tracer concentrations are low and glucose concentrations simultaneously high. It is easy to see that small errors in the concentration, will lead to greater changes in slope and even larger variations in the estimate of R_a. This is why the 'optimal segment method' of smoothing [25], which was developed specifically in the context of this calculation, astutely focuses on minimizing variations in slope.

When the metabolic clearance rate, MCR, or k vary in time, as is true in the general nonsteady-state situation, equation (6) is the only option (for a one-compartment system). In the basal situation encountered here, it was shown that MCR is constant or nearly so. This statement is critical since it might be argued that the MCR was assumed constant and the remainder of the observations simply follow. Several lines of reasoning can be used to show that the data do indeed show the relatively invariant nature of MCR under basal conditions in diabetes.

Firstly, all models are based on a series of assumptions about the way in which the system works. Equations (1) and (2), and more specifically (3), are based on the assumptions that the glucose system under basal conditions can be described by a single compartment and that the MCR (or k) is constant. The experiment then tests these assumptions. If the data behave consistently with this model, then, this lends support to the validity of the model, this means the constancy of MCR. How well the data conform to the model is then an important question. Visual inspection of the fits to data, illustrated above, helps. More objective criteria can be provided by statistical tests and alternative approaches. The 'runs test' was used to preclude the existence of trends in the residuals (differences between the observed and fitted data points) and therefore that these might indicate systematic behaviour of the data which is not accounted for by the model. Another approach was to examine the distribution of residuals against a theoretical normal (Gaussian) distribution. The resulting linear relation shown above is another demonstration of the random nature of the residuals and therefore the consistent nature of the model. Finally, allowing deviations from a constant k, and recalculating the flux rates using equation (4), showed that even when allowed, these deviations are small.

Lastly, the implications of a 'primed' tracer infusion as an experimental strategy can be explored. When tracer is infused at a constant rate (and without a primer), this leads to a tracer concentration which is indistinguishable from its asymptotic value (and therefore appears constant), after sufficient time has elapsed. Under these conditions some of the variability discussed above has been eliminated. This can, however, take a long time, particularly if k is reduced as in the diabetic state. Two strategies can be used to overcome this problem. The first is to fit the tracer data either to equation (1), or its solution (3). When this is done, spurious errors in slope are eliminated and the calculations of R_a (and R_d) show a consistent pattern for the duration of the study, as illustrated in the studies shown here. As an alternative to mathematical manipulations, one can 'prime' the system using an appropriate injection of tracer, which ideally eliminates, and at least reduces, the contribution of the time-dependent term in equation (3). Because in a single compartment system of volume V, the response to a tracer injection of I^* units is:

$C^* = \frac{I^*}{V} e^{-kt}$, using equation (3), the optimal injection occurs when $I^* = \frac{R_a^*}{k}$ (7)

If the primer is 'perfect', and C^* is constant, k can be identified from equation (7).

From equation (3), $C^* = \frac{R_a^*}{V \cdot k}$ and therefore, using equation (7), also $V = \frac{I^*}{C^*}$ (8)

The parameters of the system can therefore be identified using either experimental paradigm. This is important since a study obtained near-constant C^* using a primer adjusted according to glucose concentrations [10]. By the mathematical arguments just given, this could not have occurred unless, in those studies also, k was constant or nearly so. Independent experimental approaches are therefore consistent with the conclusion that MCR (or k) is indeed near constant under basal conditions in healthy subjects and in diabetic patients.

The implications of a constant k, and therefore (eventually) a constant C^*, can be further examined. These will be shown to lead to a simplified equation for R_a and well-defined sources of potential error in the calculation of R_a. Since, in healthy subjects (or animal models), k is about 0.01, the injection usually recommended is 100 min of infusion. In diabetes, this will cause underpriming and it has been recommended [10, 14] that the primer be increased by the ratio of the fasting glucose and 5 mmol/l or 5.5 mmol/l (normal fasting concentrations). As seen in the studies above, k was about 0.06 in diabetes and therefore any primer would need to be increased, on average, by almost 70%.

When constant C^* is reached (by any experimental manipulation), equation (6) simplifies to:

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

$$R_d + V \cdot \frac{dC}{dt} \qquad (9)$$

since MCR = R_a^*/C^* when C^* is constant. This equation shows that, once (the ~constant) MCR has been determined, R_a depends only on C and dC/dt. It also indicates that the determination of R_a is independent of the mode of tracer administration. Finally it clearly shows the other potential sources of error: if the (usually negative) gradient in C is not taken into account, R_a will be overestimated by an approximately constant amount ($V\ dC/dt$). The slope in C will vary, depending on the subject. As can be seen in the above studies, on average $V\ dC/dt$ comprised about 12 to 16% of the value of R_a, throughout the study, although in individual studies this quantity rose to 40%. The error in neglecting potential changes in glucose slope, can therefore be quite high and interestingly, amounts exactly to the difference between R_d and R_a, which drives the decline in the glucose concentrations.

The term, $V\ dC/dt$, also depends on V. This volume must also be accurately estimated. In the studies above V was estimated from the data and found to be nearly 18% of the body weight. This is almost exactly the suggested estimate used in a nonsteady state situations in dogs, which was 18.75% (25% body weight · a 'pool fraction' of 0.75, [26]) but again could vary with the subject. It should be noted that the pool fraction was used to compensate for physical gradients of glucose concentrations from the blood vessel to the cell wall where glucose is removed. In this context, it is interesting to also note that, in the above studies, V was found to vary inversely with k ($r = -0.73$, $p = 0.0014$). This logically suggests that as k decreases, the glucose gradient decreases, the circulating glucose is more representative of the interstitial concentration and therefore the effective volume of distribution increases. It would follow that in diabetic patients in general, V would be expected to be higher than in healthy subjects, since k is lower.

It has been suggested that tracer disequilibrium is responsible for errors in estimates of R_a [9,10] under nonsteady-state conditions. This implies a specific activity, or enrichment, a, which varies throughout the system. This is only a problem if the system is assumed to be a single-compartment with, by definition, uniform C, C^* and therefore, a. In fact such non-uniformity automatically implies a higher order system. Improved estimates of the glucose fluxes with higher order models are therefore possible. In general, these have been shown to track rapidly changing rates of glucose appearance better than the single compartment model [26–28]. However, under basal conditions, although these are nonsteady state, concentrations and flux rates change slowly and the system is well-described by a one-compartment model. This is indicated by the lack of improvement in fit when a two-compartment system is used. From another perspective, calculations for R_a based on a two-compartment system can be viewed as adding a further correction term to the formulas shown in equations (6) or (9) [26]. In the above calculations, this correction was less than 2% (data not shown). These observations suggest that with the slow changes in glucose fluxes and concentrations under basal conditions, the one-compartment model is sufficient and errors are due to the measurement issues outlined above. In conclusion, as long as the experimental and the modelling strategies are consistent, valid measures of glucose fluxes can be expected.

The studies presented here, show that in early diabetes, after an overnight fast (i) glycaemia is increased in the morning but declines during the day, as fasting continues and is, in general, not in steady state; (ii) this decrease is driven by a positive net uptake of glucose ($R_d - R_a$); (iii) the initial (early morning, fasting) glucose production rate, R_a, is increased relative to control subjects and this increase is physiological and not a methodological artefact (iv) after 10 h, R_a is indistinguishable from that in the control subjects (v) the glucose production rate is highly correlated to glycaemia both in time, and across the patient population; (vi) this correlation however, is only evident when glycaemia and R_a are changing and seems to correspond to the 'labile' portion of

this flux – by 22 h of fasting, plasma glucose has stabilized and the correlation is no longer apparent. What has not been emphasized is that (vii) the metabolic clearance rate of glucose did not change with time. This probably also contributes to the observation that (viii) the correlation between MCR and glycaemia which is also seen early in the study, persists for its duration.

A decreasing glucose concentration is almost uniformly found in metabolic studies in Type II diabetes [1–3, 8–11, 13, 14, 17–19], which is a nonsteady state situation. This leads to an R_d which is necessarily greater than R_a. This was found in the above studies and in some [10] but not in all studies [14]. In many studies, R_d is not reported. Of note, the difference, R_a-R_d, as shown above, remains quite constant for most of the duration of the study. This is consistent with the demonstrated linear nature of the decline in glucose concentrations, C as can be seen by rewriting equation (9) as:

$\frac{dC}{dt} = \frac{1}{V} \cdot (R_a - R_d)$. Integrating, one obtains: $C = A - Bt$, where, under these circumstances, A and B are positive constants.

These studies report that MCR was essentially constant, even as glycaemia changed. This seems to preclude acute saturation effects of glucose concentrations on MCR in these diabetic patients, as might have been anticipated from the nonlinear relation between R_d and C [29–31]. It is consistent, however, with previous estimates of the time course of MCR when they have been reported [3] and with the fact that very good priming of the tracer concentrations was possible in a similar group of patients [10,13,14]. This would only occur if k was constant in equation (7).

The constancy of MCR as well as the fact that dC/dt is also approximately constant over the range where C is decreasing, in turn leads to the linear relation between R_a and C in equation (9). Since this relation exists for each diabetic patient, it might be expected that it would be reflected in a similar relation in the population. This was the case as seen in the correlations which were developed early in the study period. Similar correlations have been found in a large number of investigations [3, 4, 6–8, 16–19] although the slopes (and methods of calculation) have varied widely. Other laboratories have, however, failed to find significant correlations, at least when glycaemia was less than about 10 mmol/l [10–14]. The reason for this might be as stated above, that the correlation between R_a and glucose concentrations dissipates with time [9] and notably as these concentrations stabilize. Here a true steady-state is approached, glucose is no longer related to R_a in time nor to R_a in the subject population. Depending on the time at which this occurs in a particular group of patients, it is quite possible that different studies could have examined different aspects of this relation, all of which would likely have been at least qualitatively valid at a particular time and in a particular population. In other words, a 'snapshot' could have provided a relatively static perspective on what is an evolving process. In contrast, MCR seems to be quite consistently [3, 11, 14, 15 but not 8] related to plasma glucose, when it is reported and could determine the limiting level to which the glucose concentration converges, as it stabilizes.

In our study, when glucose stabilizes, R_a and therefore R_d not only stabilize but become comparable to that in control subjects. This lends credence to the concept that the underlying, approximately steady-state glycaemia is determined by the need to maintain a 'normal' R_d [32–34]. Thus if MCR decreases in diabetes, as seen here, glucose concentrations increase in a compensatory fashion, to keep R_d the same as in control subjects, or:

$$R_{d,norm}(= MCR_{norm} \cdot C_{norm} = R_{d,diab}(= MCR_{diab} \cdot C_{diab})$$
$$\rightarrow C_{diab} = \frac{MCR_{norm}}{MCR_{diab}} \cdot C_{norm}$$

This could also suggest the change in MCR as the more fundamental lesion, since it does not resolve over the course of a short fast, whereas increases in R_a approach normal rates over the same time period. Impairments in both processes have been suggested as basic in the development of diabetes by comparing estimates of their rates in relatives of subjects with diabetes to subjects without a family history of diabetes [35–37]. Although this study cannot resolve the pathogenesis of hyperglycaemia, it nevertheless indicates that the more 'labile' portion seems to be related to an overproduction of glucose which declines with extended fasting. The 'asymptotic' value which is approached over this longer period of fasting, could be determined by an intrinsically depressed glucose clearance resulting from fundamental changes in the cellular machinery involved with its removal.

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