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# On the determination of basal glucose production rate in patients with Type 2 (non-insulin-dependent) diabetes mellitus using primed-continuous 3-$^3$H-glucose infusion

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**Summary.** Using primed-continuous 3-$^3$H-glucose infusion, basal glucose production rate has been reported to be 140% higher than normal or almost normal in hyperglycaemic patients with Type 2 (non-insulin-dependent) diabetes mellitus. To determine whether these markedly different results could be due to the mode of priming: fixed or adjusted, or the mode of calculation: steady state or non-steady state equations, we studied 11 patients with Type 2 diabetes (fasting plasma glucose 8–20 mmol/l). For 6 h 3-$^3$H-glucose (0.40 μCi/min) was infused preceded by a priming dose of 40 μCi (fixed priming), or 40 μCi · plasma glucose (mmol/l) · $5^{-1}$ (adjusted priming). In diabetic patients the plasma glucose concentration was not constant but declined $0.52 \pm 0.07$ mmol · $l^{-1}$ · $h^{-1}$. Furthermore, the rate of fall was correlated to the fasting plasma glucose concentration ($r = 0.90$, $p < 0.01$). Thus, the fasting state was not a steady state condition. Using adjusted priming a constant tracer steady state level was obtained within 60 min. In contrast, using fixed priming tracer steady state was not reached within 6 h. The initial tracer level was far below, and increased in time towards the steady state level observed after adjusted priming. Consequently, using Steele's equations after fixed priming, glucose production rates calculated after 90–120 min were overestimated in proportion to fasting hyperglycaemia. In conclusion: The fasting state in patients with Type 2 diabetes is not a steady state condition. Adjusted priming seems most appropriate in Type 2 diabetes. By estimating glucose production 2 h after fixed priming or assuming steady state conditions, most previous studies may have overestimated basal glucose production in Type 2 diabetes in proportion to fasting hyperglycaemia.

**Key words:** 3-$^3$H-glucose, primed-continuous tracer technique, Type 2 (non-insulin-dependent) diabetes, basal glucose turnover, glucose appearance, glucose disappearance, steady-state conditions, Steele's equations.

Elevated basal glucose production rates have been suggested as being responsible for fasting hyperglycaemia in patients with Type 2 (non-insulin-dependent) diabetes mellitus [1]. This suggestion is based on tracer studies using primed-continuous infusions of 3-$^3$H-glucose. In many of these studies basal glucose production in the diabetic patients was found to be increased in proportion to fasting hyperglycaemia. Reviewing the literature, however (Table 1), it appears that although the majority of studies have found diabetic glucose production to be markedly elevated [2–18], some studies have reported normal or almost normal rates [19–24]. Therefore, the obvious question must be, whether the basal fasting state in patients with Type 2 diabetes is a high turnover condition where glucose production rates are almost twice that of normal, or a compensated condition where glucose production rates are almost normal.

In most studies reporting elevated rates [2–4, 7–16, 18] the ratio between priming dose and constant 3-$^3$H-glucose infusion rate was constant (fixed priming), whereas in studies reporting normal or slightly elevated rates [19–24], the ratio of priming dose and constant infusion rate was adjusted to the fasting plasma glucose concentration, i.e. in hyperglycaemic subjects the priming dose was increased in proportion to fasting hyperglycaemia (adjusted priming). In some studies non-steady state equations were used because of the non-steady state behaviour of glucose and tracer concentrations, whereas in other studies steady state conditions were assumed and therefore the steady state equation used.

Therefore, to determine whether glucose production in Type 2 diabetes is markedly elevated or almost normal, it became important to evaluate whether fixed priming or adjusted priming is most appropriate in hyperglycaemic patients with Type 2 diabetes, and furthermore, whether the basal state in these patients is sufficiently close to steady state conditions to justify the use of steady state calculations, or whether non-steady state equations must be used.

In order to elucidate these methodological questions we studied 11 patients with Type 2 diabetes using fixed and adjusted priming on separate occasions. Plasma glucose and tracer concentrations were measured at 10 min intervals for 6 h, and glucose turnover rates calculated using both steady state and non-steady state equations.

## Subjects and methods

### *Subjects*

Eleven Type 2 diabetic patients with fasting plasma glucose concentrations ranging from 8 to 20 mmol/l were selected from our outpatient clinic. None of the patients had received insulin treatment. As a

**Table 1.** Published studies comparing 3-$^3$H-glucose determined hepatic glucose production (HGP) in patients with Type 2 (non-insulin-dependent) diabetes and in non-diabetic control subjects

| Plasma glucose (mmol/l) | Prime (μCi) | 3-$^3$H-glucose infusion (μCi/min) | Ratio of Prime and Infusion | EQ time (min) | Basal HGP diabetic in percent of normal | Equation | Ref. |
|---|---|---|---|---|---|---|---|
| 15.9 | 50 | 0.25 | 200 | 120 | 239[a] | Steele | 2 |
| 15.6 | 50 | 0.50 | 100 | – | 207[a] | Steele | 3 |
| 15.9 | – | – | – | 120 | 196[a] | Steele | 4 |
| 14.6 | 25–50 | 0.4–0.6 | – | 120 | 191[a] | Steele | 5 |
| 14.7 | 25–50 | 0.25–0.35 | – | > 30 | 191[a] | Steele | 6 |
| 14.2 | 25 | 0.25 | 100 | > 30 | 188[a] | Steele | 7 |
| 14.6 | 30 | 0.60 | 50 | 120 | 175[a] | Steele | 8 |
| 14.1 | 25 | 0.25 | 100 | > 30 | 169[a] | Steele | 7 |
| 11.2 | 20 | 0.20 | 100 | 120 | 169[a] | Steele | 9 |
| 12.2 | 30 | 0.30 | 100 | 180 | 168[a] | SS | 10 |
| 10.4 | 14 | 0.14 | 100 | 120 | 164[a] | Steele | 11 |
| 13.9 | 30 | 0.30 | 100 | 120–180 | 164[a] | Steele | 12 |
| 14.6 | 50 | 0.50 | 100 | 60–260 | 163[a] | Steele | 13 |
| 12.4 | 35 | 0.35 | 100 | 120–180 | 160[a] | SS | 14 |
| 10.1 | 14 | 0.14 | 100 | 120 | 156[a] | Steele | 15 |
| 10.0 | 22 | 0.22 | 100 | 120 | 153[a] | Steele | 16 |
| 18.2 | 15–35 | 0.17 | 100–200 | 120 | 144[a] | SS | 17 |
| 10.9 | 33 | 0.33 | 100 | 120 | 131[a] | Steele | 18 |
| 9.0 | adj | 0.25 | adj | 120–180 | 118[a] | SS | 19 |
| 8.8 | adj | 0.25 | adj | 120–180 | 115[a] | SS | 20 |
| 8.3 | adj | 0.25 | adj | 120–180 | 115 ns | SS | 21 |
| 10.5 | adj | 0.40 | adj | 120–180 | 110 ns | SS | 22 |
| 11.0 | adj | 0.25 | adj | 120–180 | 108 ns | SS | 23 |
| 11.0 | adj | 0.40 | adj | 120 | 105 ns | SS | 24 |

[a] $p < 0.05$ diabetic patients vs normal subjects. Adj = adjusted to fasting hyperglycaemia. EQ = Equilibration time (i.e. time from start of infusion to end of basal measurement). SS = steady state

control group seven non-diabetic subjects matched for age and relative weight were also studied. Clinical characteristics of the diabetic patients and control subjects are given in Table 2. All subjects had constant body weight for at least two months before the studies and no medication was allowed for two days before each study. The diabetic patients were without signs of diabetic neuropathy, retinopathy or nephropathy. All the subjects had normal results on screening blood tests of renal and hepatic function. The purpose and risks of the study were carefully explained to all the subjects before informed consent to participate was obtained. The protocol of the study was reviewed and approved by the regional ethical committee of the county of Copenhagen.

## *Study protocol*

The diabetic patients were studied on two occasions with a 14-day interval using fixed or adjusted priming in randomized order. The non-diabetic control subjects were studied only once since for them adjusted priming equalled fixed priming. On each occasion subjects were admitted to the department on the day before the study and fasted from 22.00 hours. Apart from voiding before the study the subjects remained supine during the study period. At 07.30 hours a catheter was inserted in an antecubital vein for infusion of 3-$^3$H-glucose and another catheter inserted retrogradely in a contralateral dorsal hand vein for intermittent collection of blood samples. This hand was placed and maintained in a heated plexiglas box [25] to obtain arterialized venous blood [26]. 3-$^3$H-glucose (New England Nuclear, Boston, Mass., USA) 300 μCi was dissolved in 320 ml 0.9% NaCl and infused at a constant rate of 0.42 ml/min (0.40 μCi/min) from 08.00 to 14.00 hours. Immediately before the start of the constant infusion a priming dose of 42 ml (40 μCi) was injected on the day of fixed priming. On the day of adjusted priming the priming dose was increased in proportion to the degree of fasting hyperglycaemia: Priming dose = 40 μCi · fasting plasma glucose concentration (mmol/l) · $5^{-1}$. The fasting plasma glucose concentration for calculation of the adjusted priming dose was measured at the bedside using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, Calif., USA). Blood samples were collected in fluoride treated tubes at 10 min intervals for determination of plasma glucose and plasma 3-$^3$H-glucose activity, and every 60 min in heparin-trasylol treated tubes for determination of plasma insulin, C-peptide and glucagon, and in ice-chilled tubes for determination of non-esterified fatty acids (NEFA). Blood samples were immediately centrifuged at 5 °C and plasma stored at – 20 °C until assay.

## *Indirect calorimetry*

Indirect calorimetry was performed using a computerized flow-through canopy gas analyser system (Deltatrac, Datex, Helsinki, Finland). Briefly, air is suctioned at a rate of 40 l per min through a canopy placed over the head of the subject. Samples of inspired and expired air are analysed for oxygen concentration using a paramagnetic differential oxygen sensor and for carbon dioxide using an infrared carbon dioxide sensor. Signals from the gas analysers are processed by the computer and oxygen consumption and carbon dioxide production are calculated and recorded once per min. The average gas exchange rates recorded over 40 min in the periods 60–120 min and 300–360 min were used to calculate rates of glucose oxidation, lipid oxidation and energy expenditure as previously described [27, 28]. In this calculation a urinary nitrogen excretion of 13 g/24 h was assumed. Before each measurement period the gas analysers were calibrated with precision gas mixtures.

## *Analytical determinations*

Glucose in plasma and urine was analysed as previously described [29]. For 3-$^3$H-glucose activity 500 μl of plasma was deproteinized with 1 ml $Ba(OH)_2$, 0.3 N and 1 ml $ZnSO_4$, 0.3 N (Sigma, St. Louis, Mo., USA), 1000 μl of the supernatant was placed in 20 ml glass scintillation vials, evaporated to dryness under compressed air to eliminate $^3$H labelled water, resuspended in 1000 μl distilled water and mixed with 10 ml Aqualuma Plus (Lumac, Schaesberg, The Netherlands). After 3 days at room temperature to allow for disappearance

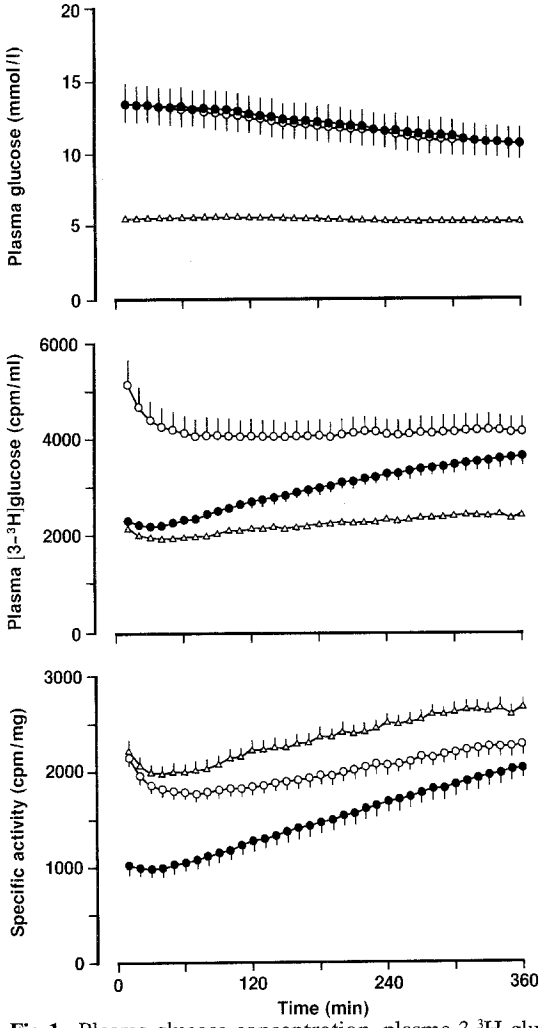


**Fig. 1.** Plasma glucose concentration, plasma 3-$^3$H-glucose activity and glucose specific activity in control subjects (-△-) and in Type 2 (non-insulin-dependent) diabetic patients during continuous infusion of 3-$^3$H-glucose (0.40 μCi/min) preceded by a fixed priming dose (40 μCi) (-●-) or an adjusted priming dose (40 μCi · plasma glucose (mmol/l) $\cdot 5^{-1}$) (-○-)

of chemoluminiscense, the vials were counted (20 min) in a liquid scintillation counter (Tri-Carb 1500, Packard Instruments, Downers Grove, Ill., USA). For the determination of infusate and background activity 500 μl of the subjects' baseline plasma with and without addition of 10 μl of the infusate solution was run through the same procedure. Both plasma, infusate and background determinations were performed in duplicate and in the same assay for a given subject. In all plasma determinations $^3$H-glucose activity was more than 20-fold above the background level. Plasma insulin [30], C-peptide [31] and glucagon [32] concentrations were measured by RIA. Plasma NEFA concentration was determined with a commercial kit using an automated analyser (Cobas Mira, Roche, Basle, Switzerland).

### *Calculations*

Glucose turnover rates were calculated at 10 min intervals using both the steady state equation [21, 33–35] and Steele's non-steady state equations [36–39] as in the previous studies.
Steady state equation:

$$Ra = Rd = \frac{Ra^*}{SA} \qquad \text{(Eq. 1)}$$

Since only one glucose turnover rate is calculated from Eq. 1, we have in this paper used the term glucose turnover rate (GT) to identify rates calculated using Eq. 1.

Steele's equations for non-steady state:

$$Ra = \frac{Ra^* - p \cdot V_D \cdot C \cdot (dSA/dt)}{SA} \qquad \text{(Eq. 2)}$$

$$Rd = Ra - p \cdot V_D \cdot \frac{dC}{dt} \qquad \text{(Eq. 3)}$$

where Ra and Rd are the rates of appearance and disappearance of unlabelled glucose, Ra* is tracer infusion rate (cpm/min), SA is glucose specific activity (cpm/mg), C is plasma glucose concentration (mg/ml), and $V_D$ is the distribution volume of glucose taken as 200 ml/kg body weight, and p is the pool fraction taken as 0.65 [40]. During each 10 min interval ($t_1$ to $t_2$) we used the approximations $C = (C_1 + C_2)/2$, $SA = (SA_1 + SA_2)/2$, $dC = (C_2 - C_1)$ and $dSA = (SA_2 - SA_1)$. In order to minimize the influence of random error the individual curves of plasma glucose and plasma 3-$^3$H-glucose activity were smoothed as proposed by Wolfe [39] using the optimal segments method [41] before turnover rates were calculated.

### *Statistical analysis*

All data are presented as mean ± SEM. Statistical comparisons between and within groups were performed using the non-parametric Mann-Whitney and Wilcoxon tests for unpaired and paired data. In correlation analysis Spearmans rho was used. $p$ values less than 0.05 were considered significant.

## Results

### *Plasma insulin, C-peptide, NEFA, and glucagon concentrations*

In diabetic patients fasting plasma insulin and C-peptide concentrations were slightly higher than normal (15 ± 2 vs 9 ± 1 mU/l, $p < 0.05$, and 0.72 ± 0.07 vs 0.54 ± 0.07 nmol/l, $p$ = NS), whereas fasting plasma NEFA (0.67 ± 0.07 vs 0.68 ± 0.06 mmol/l) and glucagon concentrations (35 ± 4 vs 30 ± 5 ng/ml) were normal. Both plasma insulin, C-peptide, NEFA and glucagon levels remained unchanged during the following 6 h study period, where the subjects continued fasting.

### *Time course for plasma glucose, 3-$^3$H-glucose activity and glucose specific activity*

The diabetic patients plasma glucose concentration was not constant, but decreased on average 0.52 ± 0.07 mmol $\cdot l^{-1} \cdot h^{-1}$ (Fig. 1), and the rate of fall in plasma glucose over the 6 h study period was correlated to the fasting plasma glucose concentration at 08.00 hours ($r = 0.90$, $p < 0.01$) (Fig. 2, left panel). In control subjects the plasma glucose concentration remained unchanged. Using adjusted priming in the diabetic patients a steady

**Table 2.** Clinical characteristics of Type 2 (non-insulin-dependent) diabetic patients and control subjects

| | Diabetic patients | Control subjects |
|---|---|---|
| Male/female | 6/5 | 3/4 |
| Age (years) | 56 ± 4 | 55 ± 3 |
| Body mass index (kg/m$^2$) | 28 ± 1 | 27 ± 2 |
| Plasma glucose (mmol/l) | 12.8 ± 1.2 | 5.1 ± 0.1[a] |
| Plasma insulin (mU/l) | 15 ± 2 | 9 ± 1[a] |
| Plasma C-peptide (nmol/l) | 0.72 ± 0.07 | 0.54 ± 0.07 |
| Diabetes duration (years) | 8 ± 2 | |

mean ± SEM, [a] $p < 0.05$

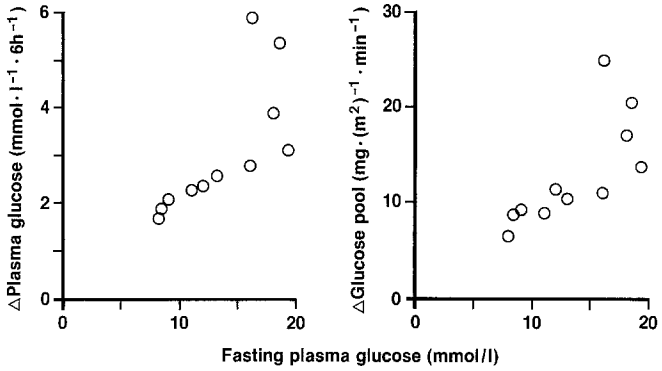


**Fig. 2.** Correlation between fasting plasma glucose concentration and (left panel) the spontaneous fall in plasma glucose concentration over the 6 h study period ($r = 0.92$, $p < 0.01$), and (right panel) the rate of change in glucose pool calculated as $V_D \cdot (dC/dt)$ ($r = 0.88$, $p < 0.01$). C = glucose concentration, $V_D$ = distribution volume (200 ml/kg)

state level of 3-$^3$H-glucose activity was obtained within 60 min (Fig. 1), and this level remained constant during the rest of the study (4114 ± 342 cpm/ml after 60 min and 4188 ± 311 cpm/ml after 360 min). In contrast using fixed priming in the diabetic patients plasma activity was much lower at 60 min (2323 ± 88 cpm/ml) and although it increased asymptotically towards the steady state level obtained after adjusted priming, it was still 12.6% below after 360 min (3659 ± 224 vs 4188 ± 311 cpm/min, $p < 0.05$). The change in average plasma activity during the last 30 min of the 2nd, 3rd, 4th, 5th and 6th hour after fixed priming was 8.2, 5.4, 5.6, 2.8 and 2.2%. In control subjects the plasma activity plateaued between 30 and 120 min, but thereafter increased slightly during the following 4 h from 2131 ± 35 to 2437 ± 62 cpm/ml. Specific activity 10 min after adjusted priming was identical in diabetic patients and control subjects, whereas using fixed priming specific activity in diabetic patients was severely reduced (1034 ± 103 vs 2164 ± 94 cpm/mg, $p < 0.01$). During the study specific activity increased reflecting the changes in plasma 3-$^3$H-glucose activity and plasma glucose concentration, simply because specific activity (cpm/mg) is plasma activity (cpm/ml) divided by plasma glucose concentration (mg/ml).

*Turnover rates in diabetic patients. Fixed vs adjusted priming*

Using the steady state equation calculated glucose turnover rates (GT) were on average 62% higher after fixed than after adjusted priming in the 90–120 min interval, whereas, at 330–360 min this difference was reduced to 18% (Fig. 3, Table 3). Also using Steele's non-steady state equations in the 90–120 min interval calculated turnover rates were on average higher after fixed than after adjusted priming (Ra: 34%, $p < 0.01$, Rd: 29%, $p < 0.01$), whereas, after prolonged tracer equilibration at 330–360 min these differences became insignificant (Ra: 2%, $p$ = NS, Rd: 4%, $p$ = NS) (Fig. 3, Table 3). Also correlations between glucose turnover rates and plasma glucose concentrations were different depending on whether fixed or adjusted priming was used (Fig. 4). After fixed priming both glucose turnover rate (GT) and Ra calculated using Steele's equation were highly correlated to plasma glucose concentration at 90–120 min ($r = 0.97$, $p < 0.001$, and $r = 0.86$, $p < 0.01$) (Fig. 4). However, as tracer steady state was approached, during prolonged infusion, these correlations decreased. At 330–360 min the correlation was still significant for glucose turnover (GT) ($r = 0.86$, $p < 0.01$) but not for Ra ($r = 0.37$, $p$ = NS). Using adjusted priming neither glucose turnover rate (GT) nor Ra were correlated to plasma glucose concentrations (Fig. 4).

*Turnover rates in diabetic patients and control subjects using adjusted priming*

Using the steady state equation glucose turnover rate (GT) was significantly elevated in diabetic patients throughout the study, (90–120 min: 92 ± 3 vs 79 ± 2, $p < 0.01$, 150–180 min: 87 ± 3 vs 75 ± 3, $p < 0.01$, and 330–360 min: 74 ± 3 vs 64 ± 3 mg·(m$^2$)$^{-1}$·min$^{-1}$, $p < 0.05$) (Fig. 3, Table 3). Almost identical values were calculated for Rd using Steele's equations (90–120 min: 93 ± 3 vs 72 ± 2, $p < 0.01$, 150–180 min: 85 ± 3 vs 70 ± 3, $p < 0.01$, and 330–360 min: 73 ± 3 vs 65 ± 3 mg·(m$^2$)$^{-1}$·min$^{-1}$, $p < 0.05$). In contrast, glucose production, Ra calculated using Steele's equations was only significantly elevated at 90–120 min (83 ± 3 vs 71 ± 3 mg·(m$^2$)$^{-1}$·min$^{-1}$, $p < 0.05$) but not at 150–180 min (76 ± 3 vs 68 ± 3 mg·(m$^2$)$^{-1}$·min$^{-1}$, $p$ = NS) or 330–360 min (67 ± 3 vs 64 ± 4 mg·(m$^2$)$^{-1}$·min$^{-1}$, $p$ = NS). Furthermore, it is seen that using non-steady state equations Ra in diabetic patients was smaller than Rd at all time points ($p < 0.01$), simply because plasma glucose concentrations were decreasing. As the rate of fall in plasma glucose concentration correlate to fasting plasma glucose concentration (Fig. 2, left panel), also the difference between Rd and Ra calculated as the rate of change in the glucose pool was correlated to fasting plasma glucose concentration ($r = 0.88$, $p < 0.01$) (Fig. 2, right panel). Calculated using the whole pool this difference averaged 12.4 ± 1.9 mg·(m$^2$)$^{-1}$·min$^{-1}$, whereas if only a fraction (0.65) of the pool is used, as in Steele's equations, this difference averaged 8.1 ± 1.2 mg·(m$^2$)$^{-1}$·min$^{-1}$.

**Table 3.** Glucose turnover rates (mg·(m$^2$)$^{-1}$·min$^{-1}$) calculated after 2, 3, and 6 h tracer infusion using steady state and non-steady state equations in control subjects and in Type 2 (non-insulin-dependent) diabetic patients using fixed (Fix) or adjusted (Adj) priming

| | | 90–120 min | 150–180 min | 330–360 min |
|---|---|---|---|---|
| Steady state equations: | | | | |
| *Glucose turnover (GT)* | | | | |
| Diabetic patients | Fix | 149.3 ± 10.9[a] | 126.4 ± 8.7[a] | 87.3 ± 4.5[b] |
| Diabetic patients | Adj | 92.0 ± 3.1 | 87.0 ± 3.0 | 74.0 ± 2.9 |
| Control subjects | | 78.7 ± 2.4[c] | 74.6 ± 2.7[c] | 64.1 ± 3.0[d] |
| Steele's non-steady state equations: | | | | |
| *Glucose appearance (Ra)* | | | | |
| Diabetic patients | Fix | 111.2 ± 7.1[a] | 96.0 ± 4.9[a] | 68.4 ± 2.7 |
| Diabetic patients | Adj | 83.0 ± 3.1 | 75.7 ± 3.2 | 67.0 ± 2.7 |
| Control subjects | | 71.2 ± 2.7[d] | 68.3 ± 3.4 | 64.3 ± 3.7 |
| *Glucose disappearance (Rd)* | | | | |
| Diabetic patients | Fix | 120.1 ± 7.7[a] | 104.2 ± 5.9[a] | 76.3 ± 2.8 |
| Diabetic patients | Adj | 92.8 ± 3.3 | 85.4 ± 3.4 | 73.2 ± 2.5 |
| Control subjects | | 72.4 ± 2.4[c] | 69.9 ± 2.9[c] | 65.2 ± 3.2[d] |

[a] $p < 0.01$ and [b] $p < 0.05$ fixed vs adjusted priming in diabetic patients, [c] $p < 0.01$ and [d] $p < 0.05$ control subjects vs diabetic patients using adjusted priming

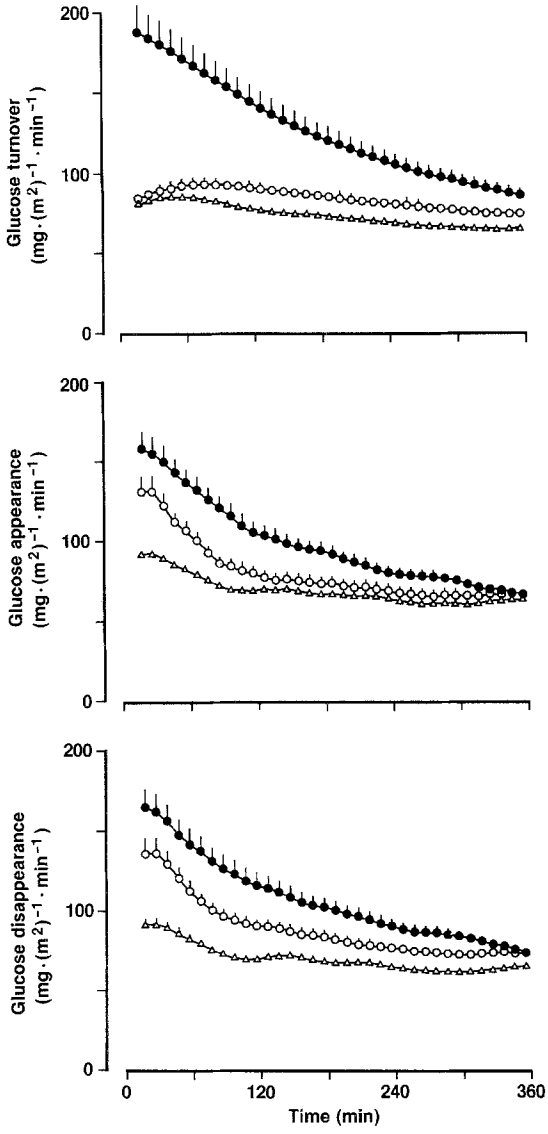


**Fig. 3.** Glucose turnover rates (GT) calculated using the steady state equation (upper panel), and glucose appearance and disappearance rates calculated using Steele's equations (middle and lower panel) in control subjects (-△-) and in Type 2 (non-insulin-dependent) diabetic patients during fixed (●) or adjusted (○) primed-continuous infusion of 3-$^3$H-glucose

Over time, from 10.00 hours (120 min) to 14.00 hours (360 min) both tracer determined Ra and Rd decreased in diabetic patients and control subjects (Table 3, $p < 0.05$). Over the same period glucose oxidation rates determined by indirect calorimetry were also reduced in both diabetic patients and control subjects ($46 \pm 4$ to $35 \pm 4$, and $48 \pm 4$ to $33 \pm 3$ mg·(m$^2$)$^{-1}$·min$^{-1}$, both $p < 0.02$), and lipid oxidation increased ($29 \pm 2$ to $35 \pm 2$, and $24 \pm 3$ to $33 \pm 2$ mg·(m$^2$)$^{-1}$·min$^{-1}$, both $p < 0.02$), whereas energy expenditure was unchanged ($0.61 \pm 0.02$ to $0.62 \pm 0.02$, and $0.58 \pm 0.02$ to $0.60 \pm 0.02$ kcal·(m$^2$)$^{-1}$·min$^{-1}$).

## Discussion

In previous studies using primed-continuous 3-$^3$H-glucose infusion to evaluate basal glucose production in patients with Type 2 diabetes the ratio of priming tracer dose and constant tracer infusion rate was either the same in all subjects (fixed priming) or adjusted for the prevalent fasting plasma glucose concentration (adjusted priming). In studies using fixed priming basal glucose production rates in diabetic patients were markedly elevated (31–139%) [2–18], whereas in studies using adjusted priming glucose production rates were normal or only slightly elevated (5–18%) [19–24]. In the present study we compared fixed and adjusted priming in 11 Type 2 diabetic subjects. Using fixed priming we found markedly higher glucose turnover rates than when using adjusted priming. Although this difference was most pronounced when the steady state equation was used, the difference was also considerable when Steele's non-steady state equations were used. Furthermore, using fixed priming glucose production rates were strongly correlated to plasma glucose concentrations, whereas using adjusted priming no correlation was found. Thus, markedly different conclusions may be reached depending on the mode of priming. Since tracer steady state was obtained 60 min after adjusted priming, but not within 6 h after fixed priming, it appears likely that turnover rates calculated using adjusted priming may be most correct, whereas turnover rates calculated after fixed priming may be in error. Furthermore, although tracer steady state could be obtained using adjusted priming, glucose concentrations in our diabetic patients were not constant but decreasing at an average rate of 0.52 mmol·l$^{-1}$·h$^{-1}$. Similar changes have been reported in other studies: 0.48 [42], 0.50 [43], 0.5 [44], 0.73 [45] and 0.52 mmol·l$^{-1}$·h$^{-1}$ [13]. Thus, the glucose system in the fasting state is not in steady state in patients with Type 2 diabetes. A decreasing glucose concentration indicates that Ra must be smaller than Rd. In previous studies using adjusted priming [19–24] the steady state equation was used and it was assumed that Ra was calculated. However, in our study, using adjusted priming glucose turnover rates (GT) calculated using the steady state equation were almost identical to Rd and higher than Ra calculated using Steele's non-steady state equations, suggesting that Rd rather than Ra may be calculated when the steady state equation is used in this situation.

Thus, our study suggests that previous studies may have overestimated basal glucose production in hyperglycaemic patients with Type 2 diabetes. Although the overestimation has been most marked in studies using fixed priming, studies using adjusted priming and steady state equations may also have overestimated glucose production to some extent. With this background it seems relevant to reconsider the basic principles of the constant infusion technique and the adequate priming dose and to try to clarify the principles of the steady state and non-steady state equations.

In principle the primed-continuous tracer infusion technique is the constant tracer infusion technique in which a priming dose of tracer is injected at the start of the infusion. With the constant infusion technique the tracer is infused at a constant rate until the tracer has ascended to a constant steady state level. Achievement of tracer steady state is important because at this constant tracer level the tracer disappearance rate (Rd*) must equal the known tracer infusion rate (Ra*).

The purpose of the priming dose is to instantaneously label the whole glucose pool to the tracer steady state level that would eventually be reached with the constant infusion alone. For a single compartment system the optimal

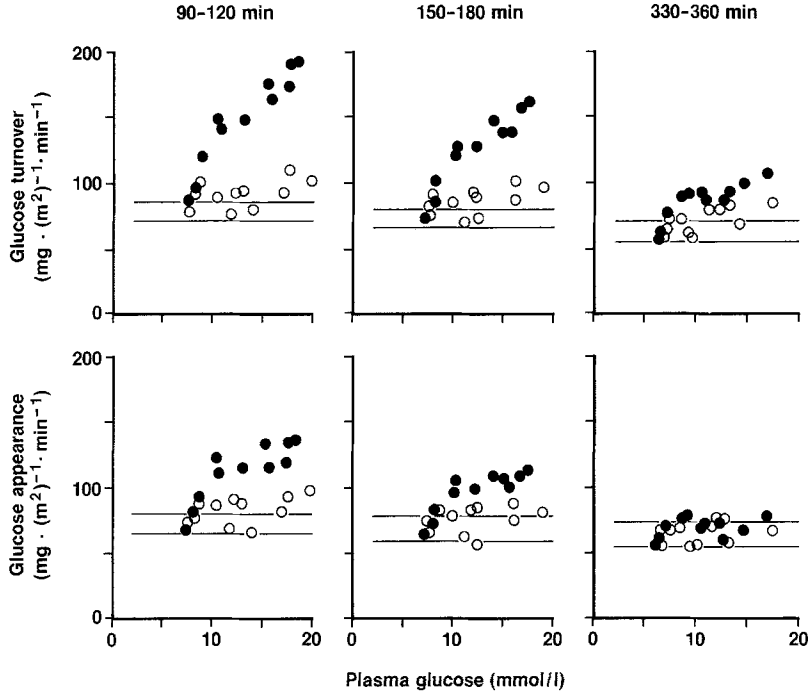


**Fig. 4.** Correlation between plasma glucose concentration and glucose turnover rate (GT) (upper panel) or glucose appearance rate calculated using Steele's equation (lower panel) after 90–120, 150–180 and 330–360 min tracer infusion in 11 Type 2 (non-insulin-dependent) diabetic patients using fixed (●) or adjusted (○) primed-continuous 3-$^3$H-glucose infusion. Mean ± SD of seven non-diabetic control subjects are indicated by horizontal lines. Using fixed priming the correlation of both glucose turnover (GT) and glucose appearance rate (Ra) to plasma glucose concentration decreased in time (GT: $r = 0.97, 0.97, 0.86$, all $p < 0.01$, Ra: $r = 0.91, 0.88$, both $p < 0.01$, and $r = 0.37$, $p$ = NS). Using adjusted priming no correlation was found (GT: $r = 0.49, 0.51, 0.46$, Ra: $r = 0.47, 0.31, 0.14$, all $p$ = NS)

ratio of priming dose and constant infusion rate depends on the mass of glucose in the pool ($V_D \cdot C$) and the rate of glucose turnover (Rd) [39]:

$$\frac{\text{Priming dose}}{\text{Constant inf.}} = \frac{V_D \cdot C}{Rd} \qquad \text{(Eq. 4)}$$

In normal subjects a ratio of 80–100 has been suggested as being appropriate [33]. Using the figures: $V_D = 200$ ml/kg, $C = 0.9$ mg/ml, $Rd = 2.0\ mg \cdot kg^{-1} \cdot min^{-1}$ a ratio of 90 is calculated. In most previous studies a ratio of 100 was used in normal subjects. In diabetic patients the glucose concentration is elevated and Rd is the unknown to be calculated. From Eq. 4 it is seen that if Rd is expected to be elevated in proportion to glucose concentration (C) so that C/Rd remains constant, the same ratio could be used for all subjects (fixed priming). However, if Rd is expected to be similar in hyperglycaemic patients and in normoglycaemic subjects, then the priming ratio must be increased in hyperglycaemic patients in proportion to the degree of hyperglycaemia (adjusted priming). Our study demonstrates that adjusted priming is more appropriate than fixed priming in patients with Type 2 diabetes because tracer steady state was obtained within 60 min using adjusted priming, whereas tracer steady state was not obtained within 6 h using fixed priming. This finding also suggests that glucose turnover rates in our Type 2 diabetic patients were not markedly elevated, but near normal.

Concerning the calculation of glucose turnover rates, Radziuk [46] recently suggested that "when glucose tracers are used to study glucose fluxes, the metabolic clearance rate (MCR) is the fundamental measurement from which Ra and Rd are themselves derived". This suggestion was based on the essential characteristics of a glucose tracer: that its kinetic and chemical behaviour is exactly analogous to that of glucose. Thus, since the glucose tracer is metabolically indistinguishable from glucose it follows that at any time point the MCR of glucose must equal the MCR of tracer:

$$MCR = \frac{Rd^*}{C^*} = \frac{Rd}{C} \qquad \text{(Eq. 5)}$$

From this fundamental tracer property an equation for Rd can be derived:

$$Rd = \frac{Rd^*}{C^*} \cdot C \qquad \text{(Eq. 6)}$$

At tracer steady state Rd* equals the known tracer infusion rate (Ra*). Therefore, at tracer steady state Rd can be calculated as:

$$Rd = \frac{Ra^*}{C^*} \cdot C \qquad \text{(Eq. 7)}$$

When glucose concentration is constant, Ra must equal Rd. However, when glucose concentration is not constant, but either increasing or decreasing, Ra is not calculated from Eq. 7. In this situation non-steady state calculations become necessary. Basically, the non-steady state equations are derived from the mass balance equations of tracer and glucose [33, 36]

$$V_D \cdot \frac{dC^*}{dt} = Ra^* - Rd^* \qquad \text{(Eq. 8)}$$

$$V_D \cdot \frac{dC}{dt} = Ra - Rd \qquad \text{(Eq. 9)}$$

Combining Eq. 6 and Eq. 8, and rearranging Eq. 9 gives:

$$Rd = \frac{[Ra^* - V_D \cdot (dC^*/dt)] \cdot C}{C^*} \qquad \text{(Eq. 10)}$$

$$Ra = Rd + V_D \cdot \frac{dC}{dt} \qquad \text{(Eq. 11)}$$

Adding the pool fraction concept advanced by Steele [36, 40] (p = effective pool fraction) we have the equations:

$$Rd = \frac{[Ra* - p \cdot V_D \cdot (dC*/dt)] \cdot C}{C*} \quad \text{(Eq. 12)}$$

$$Ra = Rd + p \cdot V_D \cdot \frac{dC}{dt} \quad \text{(Eq. 13)}$$

Mathematically, these equations are identical to Steele's equations (Eq. 2 and Eq. 3) and in practice identical rates of glucose appearance or disappearance are calculated whether these equations or Steele's equations are used. The advantage of Eq. 12 and Eq. 13 is that the measured variables, glucose concentration (C) and tracer activity (C*), are entered directly in the equations, whereas in Steele's version (Eq. 2 and 3) the combined variable, specific activity (SA = C*/C), was used. Therefore, using Eq. 12 and 13 it is easier to see how changes in tracer and glucose concentration influence the calculated rates of Ra and Rd. Furthermore, these equations underline the principle that with the tracer technique Rd is calculated from the MCR of tracer, whereas Ra is derived indirectly from Rd using the mass balance of glucose.

Using adjusted priming tracer steady state was obtained after 60 min, whereas using fixed priming a tracer steady state was not obtained. This is important, because at tracer steady state the derivative element dC*/dt equals zero, Eq. 12 is reduced to Eq. 7, and Rd can be accurately calculated independently of model assumptions. In contrast, at tracer non-steady state non-steady state equations must be used and thereby the calculated Rd values become dependent on model assumptions, i.e. instantaneous mixing throughout the whole pool and the concept of an effective pool fraction. Therefore, Rd values after adjusted priming were most correct, whereas the higher Rd values after fixed priming must represent artefactual overestimations. From Eq. 13 it is clear that when Rd is overestimated using fixed priming, Ra will also be overestimated. Figure 4 (lower panel) shows that the overestimated Ra after fixed priming was correlated to plasma glucose concentrations. However, as tracer steady state was approached during prolonged tracer infusion this correlation decreased and became insignificant after 360 min.

In some previous studies [19–24] adjusted priming was used and it was assumed that Ra was calculated from the steady state equation. Equation 7 shows that in principle Rd and not Ra is calculated from the steady state equation at tracer steady state. To calculate Ra from Rd, Eq. 9 or 13 must be used. In this context it should be realized that the rate of change in glucose concentrations was relatively small ($0.52\ mmol \cdot l^{-1} \cdot h^{-1}$), and therefore, if glucose concentration is measured only over a short period of 30 min, as in many previous studies, this trend may easily be missed. However, using the trends calculated over the 6 h study period the difference between Rd and Ra was on average $12.4 \pm 1.9\ mg \cdot (m^2)^{-1} \cdot min^{-1}$ using the whole pool, or $8.1 \pm 1.2\ mg \cdot (m^2)^{-1} \cdot min^{-1}$ if only a fraction (0.65) of the pool is used, as in Steele's equations. Furthermore, these differences were correlated to fasting plasma glucose concentrations (Fig. 2). Therefore, by assuming that Ra was calculated from the steady state equation also these studies may have overestimated glucose production in proportion to fasting plasma glucose concentrations.

The fact that most previous studies may have overestimated glucose production in proportion to fasting hyperglycaemia is important because a close correlation between fasting plasma glucose concentrations and hepatic glucose production has been used as an argument for the importance of the liver in the pathogenesis of hyperglycaemia in patients with Type 2 diabetes [1]. Also in studies of different therapies this error is important. If the therapy under study reduces the plasma glucose concentration, the mere reduction of fasting hyperglycaemia will also reduce the overestimation of glucose production. Consequently an effect of that therapy on glucose production may erroneously be assumed.

In summary tracer equilibrium in Type 2 diabetic patients is most quickly achieved when the ratio of priming dose and constant tracer infusion rate is adjusted to fasting hyperglycaemia. Using a fixed ratio more than 6 h may be required. The basal fasting state in Type 2 diabetes is not a steady state situation since plasma glucose concentration is decreasing at a rate of about $0.5\ mmol \cdot l^{-1} \cdot h^{-1}$. Therefore, by estimating glucose production 2 h after fixed priming or by assuming steady state conditions, most previous studies may have overestimated basal glucose production in Type 2 diabetes in proportion to fasting hyperglycaemia.

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