Originals

The effects of hyperglycaemia on isotopic measurement of glucose utilisation using $[2^3\text{H}]$, $[3^3\text{H}]$ and $[6^{14}\text{C}]$ glucose in patients with Type 1 (insulin-dependent) diabetes mellitus

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Summary. To determine whether hyperglycaemia alters the accuracy with which $[2^3\text{H}]$ and $[3^3\text{H}]$ glucose reflect glucose turnover measured with $[6^{14}\text{C}]$ glucose in patients with Type 1 (insulin-dependent) diabetes mellitus, glucose utilisation rates were measured during a simultaneous infusion of $[2^3\text{H}]$, $[3^3\text{H}]$ and $[6^{14}\text{C}]$ glucose after maintenance of normoglycaemia overnight and when glucose concentrations were clamped at 5.3, 7.5 and 9.7 mmol/l while insulin and glucagon concentrations were held constant. Glucose utilisation rates determined with all three isotopes were comparable in the diabetic patients at all glucose concentrations studied. On the other hand, glucose utilisation rates in nondiabetic subjects determined with $[6^{14}\text{C}]$ glucose were greater ($p < 0.01$) than

those determined with $[3^3\text{H}]$ glucose and lower ($p < 0.04$) than those determined with $[2^3\text{H}]$ glucose during the 5.3, 7.5 and 9.7 mmol/l clamps. Nevertheless, glucose utilisation rates in the diabetic patients were lower ($p < 0.05$) than those in the nondiabetic subjects for each glucose isotope. We conclude that hyperglycaemia does not alter the pattern of metabolism of $[2^3\text{H}]$ or $[3^3\text{H}]$ glucose in patients with Type 1 (insulin-dependent) diabetes mellitus.

Key words: Type 1 (insulin-dependent) diabetes mellitus, hyperglycaemia, glucose utilisation, glucose production, insulin action, $[6^{14}\text{C}]$ glucose, $[3^3\text{H}]$ glucose, $[2^3\text{H}]$ glucose, glucose production, glucose utilisation.

Tritiated isotopes of glucose are widely employed to measure glucose utilisation in human research. Their use rests on the assumption that metabolism of radioactively labelled glucose provides an accurate reflection of the metabolism of non-radioactive glucose. We recently have reported in non-diabetic man that under conditions of either hyperglycaemia [1] or hyperinsulinaemia [2], glucose turnover determined with $[2^3\text{H}]$ glucose was significantly higher than turnover measured with $[6^{14}\text{C}]$ glucose suggesting the existence of a glucose/glucose-6-phosphate cycle in normal man. In contrast, glucose turnover determined with $[3^3\text{H}]$ glucose was lower than that determined with $[6^{14}\text{C}]$ glucose suggesting the presence of an isotope effect interfering with the metabolism of $[3^3\text{H}]$ glucose. Under conditions of high turnover, $[3^3\text{H}]$ glucose but not $[6^{14}\text{C}]$ glucose underestimated the glucose infusion rate required to maintain euglycaemia thereby yielding the “negative” endogenous glucose production rates observed by numerous investigators under the same experimental conditions [2–13].

Somewhat surprisingly, $[3^3\text{H}]$ and $[6^{14}\text{C}]$ glucose provided an identical assessment of glucose utilisation in patients with Type 1 (insulin-dependent) diabetes

mellitus both in the basal state and during insulin-induced stimulation of glucose turnover. Glucose utilisation was slightly but significantly higher when determined with $[2^3\text{H}]$ glucose than $[6^{14}\text{C}]$ glucose suggesting that as in nondiabetic subjects, insulin does not alter glucose/glucose-6-phosphate cycling in diabetic patients. All three isotopes accurately reflected the glucose infusion rate required to maintain euglycaemia; glucose production rates were not negative in patients with Type 1 diabetes. These data suggested that the alteration in metabolism of $[3^3\text{H}]$ glucose that occurred during hyperinsulinaemia in non-diabetic individuals did not occur in patients with Type 1 diabetes mellitus.

To further explore this puzzling observation, the current studies were undertaken to determine whether $[3^3\text{H}]$ glucose accurately reflected glucose utilisation measured with $[6^{14}\text{C}]$ glucose in patients with Type 1 diabetes mellitus when turnover was stimulated by hyperglycaemia rather than hyperinsulinaemia. We also wished to determine whether hyperglycaemia failed to alter the rate of glucose/glucose-6-phosphate cycling in diabetic patients as it had in nondiabetic subjects.

To do so, glucose utilisation was measured during a simultaneous infusion of $[2^3\text{H}]$, $[3^3\text{H}]$ and $[6^{14}\text{C}]$ glucose

after maintenance of normoglycaemia overnight, and when glucose concentrations were varied within the physiologic range while insulin and glucagon concentrations were held constant. Results obtained in the diabetic patients were compared to those observed in non-diabetic subjects.

Subjects and methods

Following approval from the Mayo Clinic Institutional Review Board, 6 normal weight patients with Type 1 diabetes mellitus (2 male, 4 female; weight 66 ± 4 kg, body mass index 24 ± 1 kg/m²; age 38 ± 4 years), and 9 matched healthy nondiabetic subjects (6 male, 3 female; weight 69 ± 4 kg, body mass index 23 ± 1 kg/m²; age 40 ± 6 years) with no family history of diabetes mellitus, gave informed written consent to participate. Results of the studies in the nondiabetic subjects have been previously reported [1]. All diabetic patients had a history of ketosis and an absent C-peptide response to glucagon stimulation. Mean duration of diabetes was 11 ± 4 years and mean glycosylated haemoglobin at the time of study was $8.2 \pm 0.9\%$ (normal range 4–7%. Glyc-Affin, Akron, Oh, USA). None of the diabetic patients had significant retinopathy or nephropathy.

Study protocol

Diabetic patients were admitted on the day before the study having received no long- or intermediate-acting insulin that day. Patients were connected to a closed loop insulin infusion device (Biostat GCIIS, Life Science Instruments, Miles Laboratory, Elkhart, Ind, USA) [14]. At 17.00 hours plasma glucose was then rendered euglycaemic (5.0–6.1 mmol/l) and maintained euglycaemic overnight until the beginning of the hormone infusions at 09.00 hours. Nondiabetic subjects were admitted on the morning of the study.

In both groups, studies began after an overnight (12 h) fast at 07.00 hours when a primed/continuous (100/1) infusion of [⁶¹⁴C]glucose (0.13 μ Ci/min, specific activity 49.1 μ Ci/mmol), [³³H]glucose (0.26 μ Ci/min, specific activity 10.1 μ Ci/mmol) and [²³H]glucose (0.26 μ Ci/min, specific activity 24.0 μ Ci/mmol) (all isotopes New England Nuclear, Boston, Mass, USA; purity 97–99%) was commenced. After a 2-h isotope equilibration period, an infusion of somatostatin (60 ng·kg⁻¹·min⁻¹, Serono Randolph, Mass, USA), insulin (0.2 mU·kg⁻¹·min⁻¹, Actrapid Human, Squibb Novo, Princeton, NJ, USA), glucagon (0.5 ng·kg⁻¹·min⁻¹, Lilly, Indianapolis, Ind, USA) and (in two diabetic patients and eight nondiabetic subjects) growth hormone (6 ng·kg⁻¹·min⁻¹, National Pituitary Agency, University of Maryland, Baltimore, Maryland, USA) was started to maintain hormone concentrations constant under all experimental conditions. Four of the diabetic patients and one nondiabetic subject did not receive growth hormone, being studied after the withdrawal of growth hormone by the National Pituitary Agency. Variable amounts of glucose were infused to “clamp” the plasma glucose concentration at, consecutively, 5.3 mmol/l (from 0–180 min), 7.5 mmol/l (from 181–300 min) and 0.7 mmol/l (from 301–420 min).

Analytical techniques

Arterialised venous samples were used for all analyses. Plasma glucose concentrations were measured using a glucose oxidase method (YSI, Yellow Springs, Oh, USA). Plasma free insulin [14], C-peptide [15], glucagon [16] and growth hormone [17] were measured by radioimmunoassay.

The determination of plasma specific activities of [³³H] and [²³H]-glucose were determined by a modification of the method of Issekutz [18], which has been described in detail elsewhere [2]. Briefly, samples were deproteinized using Ba (OH)₂ and Zn SO₄, and subsequently passed through anion (AGI-X8, Biorad, Richmond,

Calif, USA) and cation (AG 50W X8) exchange columns. After drying under air and reconstitution in 133 mmol/l phosphate buffer, one aliquot was counted in a dual channel liquid scintillation spectrometer for determination of total [¹⁴C] and [³H] radioactivity. In a second aliquot, [²³H]glucose was selectively detritiated ($97 \pm 1\%$) without detritiation of [³³H]glucose ($99 \pm 1\%$). The residue was counted to determine [³³H]glucose radioactivity. [²³H]glucose radioactivity was determined by subtracting [³³H]glucose radioactivity from total tritium radioactivity. All calculations were corrected for completeness of detritiation using [²³H] and [³³H]glucose standards included in each assay. The third aliquot was used to correct [¹⁴C]glucose specific activity for Cori cycle activity. [¹⁴C]glucose present in the samples was selectively decarboxylated using a modification [19] of the method of Kalhan [20] to estimate the amount of radioactivity that had been randomised from the C₆ position of infused glucose to the C₁ position. Four times this amount was subtracted from total [¹⁴C]glucose radioactivity [21].

Glucose utilisation rates were determined for each isotope prior to hormone infusion and during the last 40 min at each glucose concentration. Mean values over each interval are presented in the figures and text. [¹⁴C]glucose specific activity, corrected for Cori cycle activity, was used to calculate [⁶¹⁴C]glucose turnover. Turnover rates were calculated using the equations of Steele et al. [22] as modified by DeBodo [23], assuming a nonsteady-state pool correction factor of 0.5 and a volume of distribution of 20%.

Statistical analysis

Data in the figures and text are given as mean $\pm$ SEM. Statistical analyses were performed using the Student's *t*-test with a *p* value < 0.05 considered statistically significant. Results in the subjects who did and did not receive growth hormone were similar and, therefore, were combined for statistical analysis. In order to avoid multiple comparisons, the overall mean of glucose turnover during the entire glucose clamp was determined for each isotope by summing the results determined during the 5.3, 7.5 and 9.7 mmol/l clamps and dividing by three; statistical analysis was then performed using the overall mean.

Results

Plasma glucose, insulin, C-peptide, glucagon and growth hormone concentrations (Fig. 1)

Plasma glucose concentrations in the diabetic patients and nondiabetic subjects were similar both prior to the initiation of the glucose clamps (5.3 ± 0.1 vs 5.4 ± 0.2 mmol/l) and during the consecutive glucose clamps (5.4 ± 0.1 vs 5.3 ± 0.1 mmol/l, coefficient of variation $5.1 \pm 0.6\%$ and $5.8 \pm 2.1\%$; 7.8 ± 0.1 vs 7.4 ± 0.1 mmol/l, coefficient of variation 3.1 ± 0.2 and $4.2 \pm 0.7\%$; 9.8 ± 0.1 vs 9.8 ± 0.1 mmol/l, coefficient of variation $2.9 \pm 0.5\%$ and $3.9 \pm 0.6\%$ mmol/l respectively). Plasma free insulin concentrations prior to the clamps were higher ($p < 0.001$) in the diabetic patients than in the nondiabetic subjects (6.0 ± 1 vs 3 ± 1 mU/l). During the glucose clamps, plasma free insulin concentrations in the diabetic patients were slightly higher than in the nondiabetic subjects (10 ± 1 vs 7 ± 1 , 9 ± 1 vs 7 ± 1 and 9.0 ± 1 vs 8 ± 1 mU/l, respectively, during the 5.3, 7.5 and 9.7 mmol/l clamps). The differences were only statistically different during the 7.5 mmol/l clamp ($p < 0.05$).

Fig. 1. Plasma glucose, insulin, C-peptide, glucagon and growth hormone concentrations. Somatostatin, insulin, glucagon and growth hormone infusions were started at time 0. Open circles represent results in diabetic patients and closed circles, results in nondiabetic subjects

Fig. 2. Glucose infusion rates required to maintain target glucose concentrations. Open circles represent results in diabetic patients; closed circles, results in nondiabetic subjects

Fig. 3. Isotopically determined glucose utilisation rates during the 5.3, 7.5 and 9.7 mmol/l glucose clamps. The dotted lines represent results in diabetic patients and the solid lines results in nondiabetic subjects. The open circles represent results determined with $[2^3\text{H}]$ glucose, the triangles results obtained with $[6^{14}\text{C}]$ glucose, and the closed circles results obtained with $[3^3\text{H}]$ glucose

Plasma C-peptide concentrations prior to the clamps were significantly lower ($p < 0.01$) in the diabetic patients than the nondiabetic subjects (0.2 ± 0.1

vs $0.8 \pm 0.1 \mu\text{g/l}$). During the glucose clamps, plasma C-peptide concentrations were similar in the diabetic patients and nondiabetic subjects. Values in the nondiabetic subjects were significantly ($p < 0.01$) reduced during the clamps indicating suppression of endogenous insulin release by somatostatin.

Plasma glucagon concentrations were slightly but not significantly higher in the diabetic patients than in the non-diabetic subjects both prior to (337 ± 91 vs $195 \pm 14 \text{ ng/l}$) and during the clamps (375 ± 100 vs 205 ± 16 ; 390 ± 15 vs 201 ± 15 and 340 ± 95 vs $201 \pm 15 \text{ ng/l}$ respectively). Plasma growth hormone concentrations prior to the clamps in the diabetic patients were higher ($p < 0.05$) than the non-diabetic subjects (2.5 ± 0.8 vs $0.7 \pm 0.2 \mu\text{g/l}$). During the clamps, plasma growth hormone concentrations were not significantly different in the diabetic patients vs non-diabetic subjects.

Glucose infusion rates required to maintain euglycaemia (Fig. 2)

Glucose infusion rates required to maintain euglycaemia during the final 40 min of each clamp were similar during the 5.3 mmol/l clamp (diabetic vs nondiabetic, 8.0 ± 1.2 vs $8.8 \pm 2.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) but were significantly lower in the diabetic patients during the 7.5 mmol/l (11.6 ± 1.1 vs $22.8 \pm 3.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.02$) and 9.7 mmol/l clamps (2.7 ± 0.3 vs $4.7 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.01$).

Glucose utilisation (Fig. 3, Table 1)

Postabsorptive glucose utilisation rates in the diabetic patients were similar whether determined with $[2^3\text{H}]$, $[3^3\text{H}]$ or $[6^{14}\text{C}]$ glucose. During the 5.3, 7.5 and 9.7 mmol/l clamps, glucose utilisation rates in the diabetic patients determined with $[2^3\text{H}]$ glucose, $[3^3\text{H}]$ glucose and $[6^{14}\text{C}]$ glucose also were equivalent ($p = 0.19$ or greater for overall means).

In contrast, glucose utilisation rates in the nondiabetic subjects in the postabsorptive state determined with $[6^{14}\text{C}]$ glucose were greater ($p < 0.01$) than those determined with $[3^3\text{H}]$ glucose and slightly but not significantly less ($p = 0.08$) than those determined with $[2^3\text{H}]$ glucose. Mean glucose utilisation rates during the 5.3, 7.5 and 9.7 mmol/l clamps determined with $[6^{14}\text{C}]$ glucose also were higher ($p < 0.02$, for overall mean of utilisation rates) than those determined with $[3^3\text{H}]$ glucose and lower ($p < 0.05$, for overall mean of glucose utilisation rates) than those determined with $[2^3\text{H}]$ glucose.

Postabsorptive glucose utilisation rates determined with $[2^3\text{H}]$, $[3^3\text{H}]$ or $[6^{14}\text{C}]$ glucose in diabetic patients were not significantly different from those obtained with the same isotope in the nondiabetic subjects.

The glucose utilisation rates determined with $[2^3\text{H}]$, $[3^3\text{H}]$ and $[6^{14}\text{C}]$ glucose during the 5.3, 7.5 and

Table 1. Glucose utilisation ($\mu\text{mol}/\text{kg} \cdot \text{min}$)

Diabetic patients (<i>n</i> = 6)	Pancreatic clamp and exogenous glucose infusion											
	Post-absorptive			5.3 mmol/l			7.5 mmol/l			9.7 mmol/l		
	2^3H	6^{14}C	3^3H	2^3H	6^{14}C	3^3H	2^3H	6^{14}C	3^3H	2^3H	6^{14}C	3^3H
Patient 1	14.4	12.2	12.2	13.1	12.7	12.4	20.3	20.1	18.7	23.3	17.0	21.7
2	13.3	11.2	12.7	14.5	14.7	13.6	16.8	14.1	15.5	13.3	19.7	18.9
3	13.5	11.8	12.2	13.9	12.9	13.3	19.3	17.6	17.5	22.1	18.8	20.1
4	13.2	12.9	11.2	10.3	10.2	10.1	14.8	11.9	11.7	13.2	15.7	11.7
5	10.1	10.1	10.1	8.3	9.9	9.6	12.7	13.9	13.4	15.1	20.5	16.6
6	12.1	12.4	12.8	13.8	13.2	12.3	18.5	18.3	15.3	15.6	18.3	16.7
Mean	12.8	11.8	11.9	12.3	12.3	11.9	17.1	16.0	15.4	17.1	18.2	17.6
$\pm$ SEM	0.6	0.4	0.4	1.0	0.8	0.7	1.2	1.3	1.1	1.8	0.7	1.4
Non-diabetic subjects (<i>n</i> = 9)												
Mean	15.0	13.0	11.5	18.2	16.8	15.1	28.1	26.4	24.8	30.3	28.3	26.0
$\pm$ SEM	1.0	0.6	0.7	1.5	2.0	1.5	2.5	2.7	2.2	2.6	2.7	2.3

Patients 1 and 2 received growth hormone; patients 3–6 did not. Data from the non-diabetic subjects have been previously reported [1]

Table 2. Endogenous glucose production ($\mu\text{mol}/\text{kg} \cdot \text{min}$)

Diabetic patients (<i>n</i> = 6)	Pancreatic clamp and exogenous glucose infusion											
	Post-absorptive			5.3 mmol/l			7.5 mmol/l			9.7 mmol/l		
	2^3H	6^{14}C	3^3H	2^3H	6^{14}C	3^3H	2^3H	6^{14}C	3^3H	2^3H	6^{14}C	3^3H
Patient 1	14.8	12.6	12.6	3.3	2.9	2.6	5.0	4.7	3.3	2.2	−4.1	0.6
2	14.0	11.9	13.4	4.1	4.3	3.2	3.7	1.0	2.4	−2.6	3.7	3.0
3	14.4	12.8	13.1	4.4	3.4	3.9	4.1	2.4	2.3	5.0	1.8	3.1
4	16.5	16.2	14.6	7.7	7.6	7.6	8.4	5.6	5.3	4.0	6.6	2.5
5	11.2	11.2	11.2	−1.2	0.4	0.1	0.5	1.8	1.2	0.9	6.3	2.4
6	11.9	12.2	12.6	4.7	4.3	3.3	7.4	7.2	4.2	3.4	6.1	4.6
Mean	13.8	12.8	12.9	3.8	3.8	3.5	4.9	3.8	3.1	2.2	3.4	2.7
$\pm$ SEM	± 0.8	± 0.7	± 1.4	± 1.2	± 1.0	± 1.0	± 1.2	± 1.0	± 0.6	± 1.1	± 1.7	± 0.5
Non-diabetic subjects (<i>n</i> = 9)												
Mean	15.0	13.0	11.4	9.0	7.6	5.9	5.5	3.8	2.1	4.0	2.0	−0.3
$\pm$ SEM	1.0	0.7	0.7	1.3	1.0	1.1	2.2	0.9	1.4	1.7	1.7	2.0

Data in the non-diabetic subjects have been previously reported [1]

9.7 mmol/l glucose clamps were lower in the diabetic patients than those determined with the same isotope in the nondiabetic subjects. ($[2^3\text{H}]\text{glucose}$, $p < 0.02$, 0.01 and 0.01; $[3^3\text{H}]\text{glucose}$, $p < 0.08$, 0.01 and 0.01; $[6^{14}\text{C}]\text{glucose}$, $p < 0.06$, 0.01 and 0.01 during the 5.3, 7.5 and 9.7 mmol/l clamps, respectively).

Glucose production (Table 2)

Postabsorptive glucose production rates in the diabetic patients were similar whether determined with $[2^3\text{H}]$, $[3^3\text{H}]$ or $[6^{14}\text{C}]\text{glucose}$. During the 5.3, 7.5 and 9.7 mmol/l clamps, endogenous glucose production determined with $[2^3\text{H}]$, $[3^3\text{H}]$ and $[6^{14}\text{C}]\text{glucose}$ did not differ significantly.

Postabsorptive glucose production rates in the nondiabetic subjects determined with $[2^3\text{H}]$, $[3^3\text{H}]$ or $[6^{14}\text{C}]\text{glucose}$ did not differ significantly from those observed determined with the same isotopes in the diabetic patients. Glucose production during the

5.3 mmol/l clamp was greater ($p < 0.05$) in the non-diabetic subjects when determined with $[2^3\text{H}]$ and $[6^{14}\text{C}]\text{glucose}$ and slightly but not significantly greater when determined with $[3^3\text{H}]\text{glucose}$ than rates observed with the same isotopes in the diabetic patients. Glucose production rates in the non-diabetic subjects determined with $[2^3\text{H}]$, $[3^3\text{H}]$ and $[6^{14}\text{C}]\text{glucose}$ at the 7.5 mmol/l and 9.7 mmol/l clamps did not differ from those determined at the same glucose concentration with the same isotope in the diabetic patients.

Cori cycle activity

Cori cycle activity did not differ significantly in the diabetic patients and non-diabetic subjects in the post-absorptive state (0.5 ± 0.3 vs $0.2 \pm 0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or during the 5.3 mmol/l (1.1 ± 0.0 vs $2.2 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), 7.5 mmol/l (1.1 ± 0.0 vs 2.2 ± 0.5) or 9.7 mmol/l (1.7 ± 0.6 vs $2.2 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) clamps.

Discussion

The observation that [^3H] and [^{14}C]glucose provide the same estimate of glucose utilisation during hyperglycaemia induced stimulation of glucose uptake in Type 1 diabetic patients is reminiscent of our previous report [2] of a similar relationship between isotopes during insulin-induced stimulation of glucose utilisation. This relationship is quite different from that observed in non-diabetic subjects. The cause of the underestimation of glucose turnover by [^3H]glucose in the non-diabetic subjects but not the diabetic patients is not known. As previously discussed [2], the underestimation of glucose turnover in the non-diabetic subjects cannot be explained by uptake and release of tracer by the liver, presence of tritiated but not ^{14}C labelled intermediates in plasma, error in estimation of Cori cycle activity, or limitations of the isotopic model used to calculate turnover. Preliminary studies indicate that the underestimation cannot be accounted for by incorporation of tritiated water into glucose and is equally evident when [^3H] and [$6,6^2\text{H}_2$]glucose are used as tracers [13, 24]. Taken together, these data strongly suggest that clearance of [^3H]glucose is delayed in non-diabetic subjects at an early step in metabolism (e.g. glucose transport) due to the presence of an isotope effect.

The lack of underestimate of [^{14}C]glucose flux by [^3H]glucose in the diabetic patients is of considerable interest. The current data indicate that the concordance between isotopes is not unique to insulin-induced stimulation but also is evident during glucose induced stimulation of glucose uptake. The discrepancy between results in the diabetic patients and non-diabetic subjects cannot be ascribed to lower turnover in the diabetic patients since both isotopes provided the same estimate of glucose turnover in diabetic patients at glucose utilisation rates that resulted in underestimates in the non-diabetic subjects. The results also cannot be explained by differences in correction for randomisation of the [^{14}C]glucose between groups since under the current experimental conditions, Cori cycle activity was minimal and did not differ between diabetic patients and non-diabetic subjects. The differences between the diabetic patients and non-diabetic subjects are unlikely to be attributable to the effects of the somatostatin, glucagon or growth hormone infusions since we have previously observed the same differences when none of these hormones were infused [2]. The most plausible explanation is that the process which delays the metabolism of [^3H]glucose in non-diabetic subjects is not present in patients with Type 1 diabetes mellitus. Since an isotope effect delays the metabolism of a substrate primarily by slowing its passage through a rate-limiting step [25], the current data strongly suggest that under conditions of increased glucose turnover, the rate-limiting step for glucose uptake differs in diabetic and non-diabetic man.

The possibility remains that [^{14}C]glucose also underestimates true glucose turnover in both diabetic patients and non-diabetic subjects due to an isotope effect. We believe this to be unlikely since the magnitude of an isotope effect is dependent upon the difference in mass of the atoms involved. Whereas it can be calculated that the ratio of the specific rate constants for tritium and hydrogen is 60 to 1, the ratio for C^{14} to C^{12} is only 1.5 to 1 [26]. Furthermore, under conditions of hyperinsulinaemia where endogenous hepatic glucose release is fully suppressed, [^{14}C]glucose accurately reflected the exogenous glucose infusion rate implying minimal or no delay in the clearance of [^{14}C]glucose relative to that of non-radioactive glucose.

Glucose turnover measured with [^3H]glucose also did not differ from that measured with [^{14}C]glucose in the diabetic patients during hyperglycaemia-induced stimulation of glucose utilisation. Taken together with our previous observation that [^3H]glucose only slightly overestimated [^{14}C]glucose turnover during insulin-induced stimulation of glucose utilisation [2], these data indicate that futile cycling at the level of glucose/glucose-6-phosphate is minimal in patients with Type 1 diabetes mellitus. This conclusion is in contradistinction to previous observations that glucose/glucose-6-phosphate cycling in the post-absorptive state is increased in depancreatectomized diabetic dogs [27]. Differences in species and severity of diabetes may account for differences in futile cycling.

We have previously reported that whereas glucose uptake measured with [^3H]glucose at a given insulin concentration is lower in Type 1 diabetic patients, the glucose-induced increment in glucose uptake is not [28]. The present studies again demonstrate that at a comparable level of glycaemia, glucose uptake is lower in diabetic patients than non-diabetic subjects. However, in contrast to our previous report, the design of the studies precludes comparison of the magnitude of the glucose induced increment in glucose uptake in the diabetic patients and non-diabetic subjects. Since glucose was sequentially increased while insulin concentration was maintained constant, the present studies cannot distinguish the time-dependent increase in insulin-induced glucose utilisation from the glucose induced increment in glucose utilisation. However, they clearly indicate that the increment in glucose uptake as measured by [^3H]glucose in the diabetic patients [28] cannot be ascribed to a disproportionate stimulation of glucose/glucose-6-phosphate cycling by hyperglycaemia.

Postabsorptive glucose production rates were similar in the diabetic patients and non-diabetic subjects regardless of the isotope used. These results are consistent with the observation by previous investigators that an infusion of insulin rapidly restores hepatic glucose release in Type 1 diabetic patients (measured using a single isotope of glucose) to normal [29, 30]. It is of interest that the endogenous glucose production was

significantly lower in the diabetic patients than non-diabetic subjects during the 5.3 mmol clamp. This resulted in an equivalent requirement for exogenous glucose during the 5.3 mmol/l clamp, despite significantly lower glucose utilisation rates in the former. The lower glucose production rates in the diabetic patients during the 5.3 mmol clamp are consistent with the presence of higher portal venous insulin concentrations during the somatostatin and insulin infusion than were present in the non-diabetic subjects. Thus, even though the increments in peripheral insulin concentrations during the clamp periods compared to the postabsorptive state were identical in the two groups, C-peptide concentrations during the somatostatin infusion remained unchanged in the diabetic patients, but were markedly suppressed in the non-diabetic subjects. These data suggest that in contrast to the increase in peripheral and therefore presumably portal, insulin concentration that occurred in the diabetic patients, portal insulin concentration may have even decreased slightly in the non-diabetic subjects. The current data are in agreement with previous reports indicating that a small change in portal venous insulin concentration can have a major impact on hepatic glucose production [31]. They are also consistent with the fact that equal increments in peripheral venous insulin concentrations in diabetic and non-diabetic man are not necessarily accompanied by equal increments in portal venous insulin concentrations.

In summary, glucose utilisation rates determined with [2^3H], [3^3H] and [6^{14}C]glucose are comparable in patients with Type 1 diabetes mellitus in the postabsorptive state and when glucose concentrations are varied throughout the physiologic range. Regardless of the isotope employed, glucose utilisation rates are lower in the diabetic patients than non-diabetic subjects at each glucose concentration indicating that impaired insulin action in Type 1 diabetes mellitus cannot be ascribed to differences in isotope metabolism. However, the underestimate of [6^{14}C]glucose uptake by [3^3H]glucose during hyperglycaemic-induced increase in glucose turnover that is observed in non-diabetic subjects, does not occur in patients with Type 1 diabetes mellitus.

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