Glucose Utilization in Type 1 (Insulin-Dependent) Diabetes: Evidence for a Defect not Reversible by Acute Elevations of Insulin

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Summary. It has long been assumed that replacement of insulin in insulin-deficient diabetic patients will normalise glucose utilization. In this study, glucose utilization was measured in nine long-standing, poorly controlled diabetic patients and five control subjects, matched for age (33 ± 3 versus 33 ± 2 years) and ponderal index (22.9 ± 1.3 versus 21.7 ± 1.0). Glucose uptake was measured during steady state insulinaemia in the diabetic patients and control subjects, at euglycaemia (5.5 ± 0.5 versus 5.4 ± 0.3 mmol/l, respectively) and moderate hyperglycaemia (11.8 ± 0.9 versus 10.2 ± 0.7 mmol/l, respectively). At euglycaemia with similar free insulin levels (50 ± 19 versus 43 ± 9 mU/l; $p > 0.6$), the diabetic patients utilized less glucose than the control subjects (27.8 ± 4.2 versus 56.4 ± 5.7 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p < 0.005$). During hyperglycaemia, the diabetic patients utilized almost as much glucose as the control subjects did at euglycaemia (49.9 ± 6.4 versus $56.4 \pm$

5.7 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively). In the control subjects, a 1-mmol/l rise in glucose concentration (with insulin remaining constant) resulted in a 12.3 ± 1.3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ rise in glucose utilization. In contrast, in the diabetic patients, a 1-mmol/l rise in blood glucose resulted in a rise in glucose utilization of only 3.8 ± 0.8 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($p < 0.001$), in the presence of similar concentrations of plasma insulin. This defect of glucose utilization in Type 1 diabetic patients could not be reversed by acutely raising insulin to 247 ± 23 mU/l. It is concluded that poorly controlled, insulin-dependent diabetes has a marked defect in glucose utilization that cannot be corrected by short-term hyperinsulinaemia.

Key words: Type 1 diabetes, glucose utilization, insulin resistance.

Over 40 years ago, Soskin and Levine showed that the insulin-deficient, eviscerated-depancreatized dog utilized glucose at a reduced rate [1]. However, this defect in glucose utilization returned to normal when the blood glucose concentration was very high [2]. They surmised that the absence of insulin caused a defect in glucose utilization, which would be overcome either by hyperglycaemia (i.e. by increasing the glucose gradient) or by the acute administration of insulin [2]. It has been a long-standing tacit assumption that replacement of insulin in Type 1 (insulin-dependent) diabetic patients will return glucose utilization to normal. However, evidence is presented in this paper that in long-standing Type 1 diabetic patients on conventional treatment regimens, there is a defect in glucose utilization which persists in the presence of normal free insulin levels and cannot be corrected by acute hyperinsulinaemia.

Materials and Methods

Subjects

Experiments were performed on five normal subjects (four males and one female) and nine long-standing, Type 1 diabetic patients. Table 1

shows the relevant clinical data. The control and diabetic subjects were matched for age (32.8 ± 2.0 versus 32.8 ± 3.0 years) and ponderal index (21.7 ± 1.0 versus 22.9 ± 1.3) in control and diabetic subjects respectively. The diabetic subjects had had diabetes for a mean of 17 ± 3 years and were on an average dose of 65 U of insulin/day. The patients were selected from the diabetic clinic only on the criteria of duration of diabetes of > 5 years, and absence of major diabetic complications, as assessed by absence of proliferative retinopathy, large vessel disease and proteinuria and the presence of normal plasma urea and creatinine concentrations. No special attempt was made to control their diabetes less well before the study. The mean glycosylated haemoglobin (HbA_{1c}) level at the time of the study was $13.7 \pm 0.9\%$ (normal 4.5%–8.5%). Written informed consent was obtained from all subjects, and the protocol was approved by the Ethics and Research Committee of St. Vincent's Hospital, Fitzroy.

Study Protocol

All subjects were studied after an overnight fast and at least a 30-min period of recumbency. The diabetic subjects had their last injection of long-acting insulin on the morning of the day before each study. On the morning of a study, euglycaemia was achieved in the diabetic patients, before the start of each experiment by a 2–4 h infusion of insulin (75 mU $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). Once euglycaemic, the protocol as shown in Figure 1 was commenced. During the first 60 min of each period, the glucose infusion rate was varied to maintain euglycaemia (period 1) or hyperglycaemia (period 2). In order to inhibit endogenous insulin se-

Table 1. Clinical details of the diabetic and control subjects

Diabetic subjects	Sex	Age (years)	Ponderal index weight (kg)/height ² (m)	Duration of diabetes (years)	Dose of insulin (units)
1	M	22	22	9	NPH 96 Regular 12
2	M	27	16	20	NPH 36 Regular 12
3	F	28	24	19	Monotard 30
4	F	32	21	10	Leo Retard 44 Actrapid 26
5	M	39	22	24	Leo Retard 60
6	M	23	22	6	NPH 32 Regular 12
7	M	33	24	14	Monotard 40
8	M	41	24	17	Lente 60 Actrapid 4
9	M	50	31	30	NPH 100
Mean $\pm$ SEM	7 M 2 F	33 $\pm$ 3	23 $\pm$ 1	17 $\pm$ 3	—
Control subjects	4 M 1 F	33 $\pm$ 2	22 $\pm$ 1	—	—

cretion in the control subjects, somatostatin (Bachem, California, USA) was infused at 7.5 $\mu\text{g}/\text{min}$. No somatostatin was necessary for the diabetic patients, as they had essentially no endogenous insulin (fasting C-peptide: 0.015 ± 0.003 nmol/l compared with control subjects 0.30 ± 0.044 nmol/l; Table 2). Steady state plasma insulin levels were maintained throughout each study by fixed rates of insulin infusion (Table 2) ranging between 30–300 $\text{mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Actrapid MC, Novo Laboratories, Sydney, Australia). In all studies, glucose utilization was measured at steady state, during euglycaemia (period 1), and moderate hyperglycaemia (period 2) (Fig. 1). Plasma glucose levels were adjusted by performing modified [3] euglycaemic and hyperglycaemic clamps [4]. During the hyperglycaemic period, urine was collected for measurement of urinary glucose loss.

All solutions were infused using Gilson Minipuls II pumps (Gilson, Villiers le Bel, France) via a 20 cm catheter placed in an antecubital vein. Blood samples were collected at appropriate intervals (Fig. 1) from an indwelling butterfly needle placed in a contralateral arm vein. To overcome the problems of hormone adsorbing to the IV apparatus, insulin was pre-run for 1 h before use. Somatostatin was infused with 0.2% human serum albumin (Beringwerke, Mannheim, FRG) and 10000 KIU/ml of aprotinin (Trasylol, Bayer Pharmaceuticals, Sydney, Australia). Blood for hormone estimation was collected in 10% aprotinin, promptly centrifuged and plasma stored at -20°C .

In all diabetic subjects, the contribution of hepatic glucose production to the total rate of appearance of glucose was measured using a primed constant infusion of [$3\text{-}^3\text{H}$]-glucose [5, 6]. The priming dose of tracer varied between 15–35 μCi depending on the fasting plasma glucose (Sönksen P, personal communication). In the normal subjects, hepatic glucose production was assumed to be insignificant ($< 2.2 \pm 0.8$ $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) at the insulin infusion rates employed ($25 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) [5, 7]. In these subjects the rate of appearance of glucose equalled the rate of glucose infused intravenously.

Glucose was measured using a Union Carbide Centrifichem C400 analyser (Union Carbide Corporation, New York, USA) employing the hexokinase method. Plasma insulin (IRI) was estimated by radioimmunoassay employing dextran-charcoal separation of bound and free fractions [8]. Assay sensitivity for IRI was 1 mU/l with an interassay coefficient of variation at 10.5 mU/l of 6.3% and coefficient of variation at 22.0 mU/l of 8.4%. In the diabetic subjects, free insulin

Fig. 1. Schematic diagram of the protocol used. Somatostatin was used only in the control subjects. The bolus dose ($\blacktriangle$) of [$3\text{-}^3\text{H}$]-glucose at the start of the study varied between 15–35 μCi depending on the fasting blood glucose level. During the first 60 min of each period, glucose infusion rate was varied to maintain euglycaemia (period 1) or hyperglycaemia (period 2). The arrows ($\uparrow$) indicate sampling times

was measured as described by Nakagawa et al. [9]. The correlation coefficients (r) between the insulin infusion rates and the plasma insulin levels achieved at plateau, in diabetic subjects with circulating insulin antibodies and in normal subjects without insulin antibodies, were 0.94 and 0.97 respectively. C-peptide was estimated using the Novo C-peptide assay kit employing synthetic human C-peptide and guinea pig anti-human C-peptide antiserum [10]. Assay sensitivity for C-peptide was 0.01 nmol/l with an interassay coefficient of variation at 0.29 nmol/l of 11.4% and at 0.026 nmol/l of 24.6%. Non-esterified fatty acids (NEFA) were measured using a modification of the method of Carruthers and Young [11] and β -hydroxybutyrate levels were estimated as described by Williamson et al. [12]. The relevant plasma samples from the [$3\text{-}^3\text{H}$]-glucose tracer studies were processed as described previously [3].

Calculations

In the diabetic patients, the rate of appearance of glucose (R_a) at steady state = F/SA where F = the rate of infusion of [$3\text{-}^3\text{H}$]-glucose and SA = the specific activity of the tracer. The mean coefficient of variation for tracer counts at plateau was $3.6 \pm 0.9\%$. In three studies, steady state was not achieved (Table 2). In these, the non-steady state equation of Steele was used with 0.65 as the pool fraction [13]. In the control subjects, the rate of appearance of glucose at steady state equals the rate of glucose infused IV, since hepatic contribution to the rate of appearance of glucose is insignificant at the insulin infusion rates used [5, 7]. At steady state, plasma glucose is constant, defined as a coefficient of variation of plasma glucose concentration about plateau of $< 10\%$. Mean coefficients of variation of glucose values at steady state were $4.5 \pm 0.7\%$ for the euglycaemic and $4.2 \pm 0.7\%$ for the hyperglycaemic periods. At steady state, the rate of appearance of glucose equals the rate of disappearance (R_d) and the rate of tissue glucose utilization (R_u) equals R_d minus urinary glucose loss. Urinary glucose loss during hyperglycaemia was < 0.2 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in control subjects and 1.9 ± 0.5 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the diabetic subjects.

Results

Table 2 gives the data for plasma glucose, insulin infusion rates, free insulin and C-peptide levels, basally and

Table 2. Data in the diabetic and control subjects

Subject	Plasma glucose (mmol/l)	Insulin infusion rate (mU·kg ⁻¹ ·h ⁻¹)	Plasma free insulin (mU/l)	C peptide (nmol/l)	Rate of glucose utilization (μmol·kg ⁻¹ ·min ⁻¹)
<i>Diabetic patients</i>					
Low dose insulin infusion					
1 Basal	14.5		6	0.023	—
E clamp	6.2		17	0.022	22
H clamp	11.9	30	20	0.022	30
2 Basal	17.6		17	0.022	—
E clamp	7.7		16	0.015	44
H clamp	15.9	30	21	0.022	59
3 Basal	19.0		0	0.033	—
E clamp	5.7		23	0.025	27
H clamp	14.9	30	20	0.025	55
4 Basal	14.5		0	0.003	—
E clamp	4.5		24	0.003	16
H clamp	8.7	30	25	0.003	36
5 Basal	3.1		13	0.015	—
E clamp	3.7		21	0.013	13
H clamp	10.8	30	24	0.015	36
6 Basal	16.5		8	0.005	—
E clamp	5.8		65	0.005	16
H clamp	9.2	75	68	0.005	36
7 Basal	27.0		2	0.002	—
E clamp	7.1		46	0.002	40
H clamp	13.7	75	47	0.004	45
8 Basal	6.5		9	0.030	—
E clamp ^a	4.0		87	0.040	46
H clamp	9.5	75	84	0.025	92
9 Basal	15.8		5	0.004	—
E clamp	5.1		79	0.003	26
H clamp	11.5	75	81	0.004	60
High dose insulin infusion					
1 Basal	20.0		6	—	—
E clamp ^a	3.5		303	—	37
H clamp	10.2	300	310	—	56
3 Basal	22.8		0	—	—
E clamp	4.6		238	—	38
H clamp	10.4	250	213	—	65
4 Basal	19.2		0	—	—
E clamp	5.5		185	—	29
H clamp	11.4	300	137	—	68
6 Basal	17.0		12	—	—
E clamp ^a	5.2		295	—	44
H clamp ^a	12.8	300	308	—	78
<i>Control subjects (n = 5)</i>					
Basal	4.8 ± 0.1		5.5 ± 0.8	0.3 ± 0.04	—
E clamp	5.4 ± 0.3		49 ± 15	0.03 ± 0.01	56 ± 5.4
H clamp	10.2 ± 0.7	47 ± 8	50 ± 19	0.08 ± 0.03	114 ± 7.0

E clamp = euglycaemic clamp, H clamp = hyperglycaemic clamp. ^a In these studies, steady state conditions were not achieved.

during each of the two study periods, together with the corresponding rates of utilization of glucose. Figure 2A shows the mean rates of whole body glucose utilization during euglycaemia (5.4 ± 0.3 versus 5.5 ± 0.5 mmol/l) and moderate hyperglycaemia (10.2 ± 0.7 versus 11.8 ± 0.9 mmol/l; $p > 0.20$ in control and diabetic subjects respectively; Table 2). During euglycaemia, with comparable plasma free insulin concentrations (50 ± 19 versus 43 ± 9 mU/l; $p > 0.60$ in control and diabetic subjects respectively; Fig. 2A), the control subjects utilized twice as much glucose as the diabetic patients (56.4 ± 5.7 versus 27.8 ± 4.2 μmol·kg⁻¹·min⁻¹; $p < 0.005$). Only

Fig. 2. Rate of glucose utilization (panel A) and the change in glucose utilization per unit rise in plasma glucose (panel B) in control subjects ($n = 5$) and diabetic subjects ($n = 9$) during euglycaemia and hyperglycaemia. Insulin levels in control subjects were 50 ± 19 mU/l ($\square$) and in diabetic subjects 43 ± 9 ($\square$, $n = 9$) and 247 ± 23 mU/l ($\square$, $n = 4$). * $p < 0.005$ and ** $p < 0.001$ compared with the control group

during moderate hyperglycaemia did the diabetic patients utilize as much glucose as the control subjects at euglycaemia (56.4 ± 5.7 versus 49.9 ± 6.4 μmol·kg⁻¹·min⁻¹; $p > 0.40$, Fig. 2A). However, it was again evident that the diabetic patients used very much less glucose than control subjects at hyperglycaemia (49.9 ± 6.4 versus 114 ± 7.0 μmol·kg⁻¹·min⁻¹; $p < 0.005$) despite similar free insulin levels in control and diabetic subjects (Fig. 2A).

To examine further the influence of plasma glucose concentration on the rate of tissue glucose utilization in the two groups, a comparison was made of the change in the rate of glucose utilization in response to a 1-mmol rise in plasma glucose concentration, as shown in Fig. 2B. This demonstrated the dramatically greater increase in the rate of glucose utilization in response to hyperglycaemia in controls compared to diabetics (12.3 ± 1.3 versus 3.8 ± 0.8 μmol·kg⁻¹·min⁻¹·mmol⁻¹; $p < 0.001$) in the presence of similar concentrations of plasma insulin (50 ± 19 versus 43 ± 9 mU/l in control and diabetic subjects respectively).

To determine whether higher insulin levels could reverse the defect in glucose utilization present in the diabetic patients, experiments were repeated at higher insulin infusion rates in four of the diabetic subjects (Nos. 1, 3, 4, 6; Table 2). Figure 2A shows the results obtained in these subjects at high levels (247 ± 23 mU/l) insulin, compared to control subjects at low insulin levels (50 ± 19 mU/l). When euglycaemia is maintained (4.8 ± 0.5 mmol/l) during high dose insulin experiments, the rate of glucose utilization rose significantly in the diabetic patients to 37.0 ± 3.1 μmol·kg⁻¹·min⁻¹; $p < 0.005$ compared to the rate of glucose utilization obtained in diabetic subjects at low insulin levels (27.8 ± 4.2 μmol·kg⁻¹·min⁻¹), but remained below the rate of utilization measured at the low insulin levels in normal subjects (56.4 ± 5.7 μmol·kg⁻¹·min⁻¹; $p < 0.05$;

Fig. 2A). During hyperglycaemia (10.7 ± 0.4 mmol/l) and the high insulin study, the rate of glucose uptake in the diabetic patients was $67 \pm 5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ which was significantly greater than that obtained in these diabetic subjects during the low insulin infusion ($27.8 \pm 4.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $p < 0.01$; Fig. 2A). When the data are examined as the rate of change of glucose uptake per 1 mmol rise in plasma glucose at high insulin levels (Fig. 2B), there was no significant improvement in the efficiency of glucose uptake in response to hyperglycaemia ($4.8 \pm 0.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{mmol}^{-1} \cdot \text{l}^{-1}$) compared to glucose utilization at low insulin levels ($3.8 \pm 0.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{mmol}^{-1} \cdot \text{l}^{-1}$; $p > 0.40$; Fig. 2B). The rate of change of glucose utilization in the diabetic subjects remained strikingly below that observed for control subjects at low insulin levels (Fig. 2B).

From previous studies it could be assumed that with the insulin infusion rates used in the control subjects, hepatic glucose production would be insignificant [3, 5]. This assumption could not be made for the diabetic patients and hepatic glucose production was measured in all the diabetic subjects. Hepatic glucose production was found to be low during both the euglycaemic and hyperglycaemic periods (3.5 ± 1.7 and $3.0 \pm 1.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ respectively, $p > 0.50$), and thus comprised $< 17\%$ and $< 11\%$ of overall glucose utilization in the diabetic subjects at euglycaemia and hyperglycaemia, respectively.

Following the attainment of euglycaemia and during the euglycaemic period (period 1, Fig. 1), NEFA levels were comparable in the two groups (0.36 ± 0.04 mmol/l (range 0.27–0.39) versus 0.46 ± 0.21 mmol/l (range 0.2–0.6) in control and diabetic subjects respectively; $p > 0.60$). Similarly, β -hydroxybutyrate levels were high in the diabetic patients before the start of the study (0.77 ± 0.24 mmol/l), but fell to well within the normal range during the euglycaemic period (period 1; 0.04 ± 0.02 mmol/l (range 0.01–0.09 mmol/l); normal subjects < 0.17 mmol/l).

Discussion

This paper presents evidence that long standing, conventionally treated, insulin-requiring diabetic patients have a defect in glucose disposal, even in the presence of normal (Fig. 2) or high (Fig. 3) free insulin levels. Previous studies of glucose disposal in Type 1 diabetes are conflicting. Ginsberg could find no evidence of such a defect in a group of 12 newly diagnosed insulin dependent diabetics [14], whereas DeFronzo et al. have presented evidence of reduced glucose disposal in patients with long standing disease [15]. These findings, together with those described in this paper, suggest that the abnormality in glucose disposal develops *after* the onset of the illness and may thus be a consequence of the diabetic state itself.

The reason for the presence of this defect in glucose disposal is unclear. Studies in vivo using the euglycaemic clamp technique, measure the rate of total body glucose utilization [4, 7]. A decrease in glucose utilization can be related to an abnormality of glucose transport across the plasma membrane only if transport is the rate-limiting step. Similarly, an intracellular defect in a step of glucose metabolism, if rate-limiting, could also result in decreased glucose utilization. Thus, the reduced in vivo glucose uptake demonstrated in this study in the diabetic subjects, may be due either to a defect in glucose transport [16–18] and/or a defect in glucose metabolism [16, 18–20]. The diabetic subjects in this study were poorly controlled (as demonstrated by high HbA_{1c} levels) and were on large doses of insulin (Table 1). Therefore these patients had adequate free insulin levels for most of the day [18], and the defect of glucose utilization in the diabetic subjects was not due to chronic insulin deficiency. Interestingly, in vitro glucose metabolism has also been reported to be reduced in adipocytes isolated from Type 1 diabetic patients [18]. An alternative explanation could be that elevated NEFA or ketone bodies, factors known to interfere with glucose metabolism and insulin action [21, 22], contributed to the decrease in glucose utilization. However, both metabolites were normal in the diabetic subjects during the euglycaemic and hyperglycaemic sampling periods. Whatever the mechanism, it is clear that glucose utilization is abnormal in established insulin-dependent diabetes, even in the presence of adequate to high free insulin levels. It would be of considerable clinical interest to know if a sustained period of good diabetic control employing more physiological continuous insulin in delivery systems could reverse this defect.

The importance of the diminished glucose utilization to the pathogenesis of the hyperglycaemia in insulin-dependent diabetes is highlighted by the hepatic glucose production data. Glucose production was readily suppressed by insulin levels of around 40 mU/l, which suggests that hepatic sensitivity to insulin is normal [7] and that glucose over-production does not contribute to the glucose intolerance of these insulin-treated diabetic subjects.

In this study somatostatin was used only in the control subjects because our Type 1 diabetic patients did not secrete insulin in response to hyperglycaemia (Table 2). It has been previously shown that somatostatin per se, has no direct effects on in vivo or in vitro glucose production or utilization, apart from its effects through changes in insulin and glucagon levels [23–26]. In the present protocol insulin was infused in both groups, but glucagon was not replaced. However, glucagon does not alter peripheral glucose utilization [27] and therefore differences in glucagon concentrations between control and diabetic subjects would not have affected peripheral glucose utilization.

Finally, these data confirm that, in normal individuals, the removal of glucose from plasma post-prandially

is due to the combined effects of hyperglycaemia and a modest rise in insulin [28]. In contrast, in our diabetic patients, hyperglycaemia resulted in only a small rise in the rate of glucose uptake, despite the comparable degree of hyperinsulinaemia to control subjects (Figs. 2A and B). In fact, the defect in glucose utilization was not corrected by acute, large elevations of plasma free insulin (Fig. 2A). The latter abnormality would result therefore in higher and more prolonged elevations of blood glucose occurring post-prandially in many diabetic subjects, regardless of the circulating free insulin levels.

It is concluded that poorly controlled insulin-dependent diabetics have a marked defect in glucose utilization that cannot be corrected by short-term hyperinsulinaemia. Further studies are required to determine whether this defect in glucose disposal persists after a period of strict metabolic control.

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