Contribution of glucose/glucose 6-phosphate cycle activity to insulin resistance in Type 2 (non-insulin-dependent) diabetes mellitus

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Summary. It has been suggested that increased glucose/glucose 6-phosphate substrate cycling impairs net hepatic glucose uptake in Type 2 (non-insulin-dependent) diabetes mellitus and contributes to hyperglycaemia. To investigate glucose/glucose 6-phosphate cycle activity and insulin action in Type 2 diabetes we studied eight patients and eight healthy control subjects, using the euglycaemic glucose clamp and isotope dilution techniques with purified [2-³H]- and [6-³H] glucose tracers, in the post-absorptive state and eight patients and five healthy control subjects during consecutive insulin infusions at rates of 0.4 and 2.0 mU·kg⁻¹·min⁻¹. [2-³H]glucose and [6-³H]glucose radioactivity in plasma samples were determined using selective enzymatic detritiation, allowing calculation of glucose turnover rates for each isotope, the difference being glucose/glucose 6-phosphate cycling. Endogenous glucose production ([6-³H]glucose) was greater in diabetic than control subjects in the post-absorptive state (15.6 ± 1.5 vs 11.3 ± 0.4 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.05$) and during the 0.4 mU insulin infusion (10.1 ± 1.3 vs 5.2 ± 0.3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.01$) indicating hepatic in-

sulin resistance. Glucose/glucose 6-phosphate cycling was significantly greater in diabetic than in control subjects in the post-absorptive state (2.6 ± 0.4 vs 1.6 ± 0.2 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.05$) but not during the 0.4 mU insulin infusion (2.0 ± 0.4 vs 2.0 ± 0.3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). During the 2.0 mU insulin infusion endogenous glucose production was suppressed to a similar degree in both groups (2.6 ± 0.5 vs 3.4 ± 0.7 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) but glucose disappearance was lower in the diabetic subjects (30.8 ± 2.0 vs 52.4 ± 4.6 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.01$). During the 2.0 mU insulin infusion glucose/glucose 6-phosphate cycling was greater in the diabetic subjects (3.8 ± 0.7 vs 0.8 ± 0.6 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.05$). In conclusion, both hepatic and peripheral insulin action are impaired in Type 2 diabetes. Increased glucose/glucose 6-phosphate cycling is seen in the post-absorptive state and also during marked hyperinsulinaemia, when insulin resistance is predominantly due to reduced peripheral tissue glucose uptake.

Key words: Glucose cycle, insulin resistance, Type 2 (non-insulin-dependent) diabetes mellitus.

Increased endogenous (hepatic) glucose production (EGP) in the fasting state and under hyperinsulinaemic conditions are well recognised features of Type 2 (non-insulin-dependent) diabetes mellitus [1–4] representing resistance to the effect of insulin on the liver. Following hepatic uptake, glucose is converted by the enzyme glucokinase to glucose 6-phosphate which is an important metabolic crossroads [5]. Instead of undergoing further metabolism however, newly-formed glucose 6-phosphate may be converted back to glucose by the enzyme glucose 6-phosphatase and released to the plasma. This pathway constitutes the glucose/glucose 6-phosphate (G/G6P) substrate cycle [6].

Efendic et al. [7] have recently reported increased G/G6P cycle activity in Type 2 diabetes both before and after an intravenous glucose load. They postulated that in Type 2 diabetes, increased post-ab-

sorptive cycling limits hyperglycaemia resulting from excessive EGP, but when the direction of hepatic glucose flux is reversed from glucose production to glucose uptake (e.g. under hyperglycaemic, hyperinsulinaemic conditions), increased cycle activity decreases net glucose uptake and contributes to hyperglycaemia. Increased G/G6P cycle activity, contributing to hyperglycaemia under hyperinsulinaemic conditions, could therefore be interpreted as a form of hepatic insulin resistance.

The aims of the present study were to investigate the G/G6P cycle and its contribution to insulin resistance in Type 2 diabetes. Experiments were performed in the post-absorptive state and also during partial and maximal suppression of EGP by insulin. The tracer infusion strategy employed was designed so as to avoid negative errors in the determination of EGP [8].

Table 1. Characteristics of the Type 2 diabetic and control subjects (mean $\pm$ SEM)

	Diabetic subjects	Control subjects
Sex (male/female)	5/3	5/3
Age (years)	54 $\pm$ 1	52 $\pm$ 2
BMI (kg/m ²)	26.1 $\pm$ 0.6	25.6 $\pm$ 1.1
Systolic blood pressure (mm Hg)	150 $\pm$ 3	138 $\pm$ 4
Diastolic blood pressure (mm Hg)	84 $\pm$ 2	82 $\pm$ 2
Fasting plasma glucose (mmol/l)	10.7 $\pm$ 1.4	5.4 $\pm$ 0.0
Fasting serum insulin (mU/l)	9.0 $\pm$ 1.0	6.6 $\pm$ 0.8
Fasting serum triglyceride (mmol/l)	1.8 $\pm$ 0.2	1.3 $\pm$ 0.4
Fasting serum cholesterol (mmol/l)	6.0 $\pm$ 0.2	5.0 $\pm$ 0.4

Subjects and methods

Subjects

Eight patients with previously diagnosed Type 2 diabetes and eight age-, sex- and weight-matched healthy subjects with no family history of diabetes were studied (Table 1). All of the diabetic subjects and five of the control subjects were studied in the post-absorptive state and following insulin infusion. Three of the control subjects participated in a previous study employing a different insulin infusion protocol [9] and in these cases only the results for the post-absorptive state are used.

All subjects were less than 125% of ideal body weight (based on medium frame individuals from the Metropolitan Life Insurance tables 1956), and renal and hepatic function were normal. Blood pressure, measured after 15 min in a supine position was less than 160/90 mm Hg in all cases. Fasting total serum cholesterol and triglyceride in all subjects were less than 6.5 and 2.5 mmol/l, respectively. Of the diabetic subjects, two were taking sulphonylureas and six were being treated by diet alone. Oral hypoglycaemic agents were discontinued 2 weeks before study. Approval for the study was granted by the Ethical Committee of the Queen's University of Belfast and written and informed consent was obtained from each subject.

Tracer infusion strategy

Equal quantities (150 μ Ci) of [2-³H]- and [6-³H]glucose tracers (NET 238C and NET 100C; New England Nuclear Research Products Division, Dupont UK Ltd., Stevenage, Herts., UK), HPLC-purified by the manufacturer prior to delivery, were added to a small volume of 0.9% NaCl. The purity of the tracers was checked in our laboratory as described previously [8]. The combined tracer solution was then subdivided to provide the primed continuous tracer infusion and also to label the exogenous 20% glucose infusion in order to approximate the predicted plasma glucose specific activity ([6-³H]glucose) after 2 h of primed continuous infusion (basal specific activity). On the morning of the clamp study, before commencement of the continuous tracer infusion, blood was taken and fasting plasma glucose concentration determined at the bedside. This measurement was used to calculate the basal specific activity according to the formula described by Levy et al. [10].

$$\text{Predicted glucose specific activity (dpm}/\mu\text{mol}) = \frac{\text{Tracer infusion rate (dpm/min)}}{12 \times \text{IBW} \times (0.1G + 0.349)}$$

where IBW is percent ideal body weight and G is the fasting plasma glucose concentration.

This formula was derived from the well-known formula, specific activity = isotope infusion rate/glucose production rate. From earlier studies, Levy assumed all subjects would have a glucose production rate of 12 μ mol $\cdot$ kg IBW⁻¹ $\cdot$ min⁻¹. On the basis of experimental results testing the success of the formula, the correction factor for plasma glucose concentration G (0.1G + 0.349), derived from linear regression analysis, was incorporated.

The remaining combined tracer solution was then added to the required volume of 20% glucose solution in order to produce an exogenous glucose infusion with glucose specific activity equal to predicted basal specific activity.

Euglycaemic clamp studies

At 08.00 hours, the primed (24 $\pm$ 3 μ Ci) continuous combined infusion of [2-³H]- and [6-³H]glucose (0.24 $\pm$ 0.03 μ Ci/min) was commenced. After 2-h isotope equilibration, insulin (Humulin S; Eli Lilly and Co. Ltd., Basingstoke, Hants., UK) was administered at 0.4 mU $\cdot$ kg⁻¹ $\cdot$ min⁻¹ (chosen to produce partial suppression of EGP) and 2.0 mU $\cdot$ kg⁻¹ $\cdot$ min⁻¹ (chosen to produce maximal suppression of EGP) for consecutive 2-h periods. Arterialized blood samples were obtained at 5-min intervals for glucose estimation from a cannula (21 Venflon; Viggo AB, Helsingborg, Sweden) placed retrogradely in a dorsal vein of the right hand, which was maintained at 55°C in a thermostatically controlled perspex enclosure (Automation Division, Ashby Institute, The Queen's University of Belfast). Samples for plasma glucose were centrifuged immediately at the bedside and analysed using a glucose oxidase method (Beckman Glucose Analyser II; Beckman RIIC Ltd., High Wycombe, Bucks., UK). Plasma glucose concentration during hyperinsulinaemia was maintained at 5.3 mmol/l by the labelled exogenous 20% glucose infusion. In the diabetic subjects, plasma glucose was allowed to decline to 5.3 mmol/l before exogenous 20% glucose was given.

Samples for determination of plasma glucose specific activity were taken at 10-min intervals from -30 to 0 min before the start of insulin infusion and from 90 to 120 min and 210 to 240 min during hyperinsulinaemia, collected in iced lithium heparin fluoride tubes and separated within 15 min. Samples for insulin, non-esterified fatty acids (NEFA), glycerol and B-hydroxybutyrate were collected in plain glass tubes. Samples for pyruvate and lactate were collected in plain glass tubes containing an equal volume of 8% perchloric acid. Samples were separated as soon as clotting was complete. All samples were stored at -20°C until analysis.

Analytical techniques

Plasma for glucose specific activity was deproteinised using Ba(OH)₂ and ZnSO₄ by the method of Somogyi [11]. After centrifugation, the supernatant was passed sequentially through anion (AG1-X8; Biorad Laboratories, Watford, Herts., UK) and cation (AG50W-X8) exchange columns. The eluate was lyophilised and reconstituted in 500 μ l of 133 mmol/l phosphate buffer (pH 7.4). A modification [9] of the selective enzymatic method of Issekutz [12], which results in detritiation of [2-³H]glucose without detritiation of [6-³H]glucose, was used to determine separately [2-³H]- and [6-³H]glucose radioactivity in each plasma sample. Aliquots (200 μ l) of reconstituted plasma extract were mixed in glass liquid scintillation vials with 500 μ l of a buffered solution containing 1.2 IU hexokinase, 5 IU phosphoglucose isomerase, MgCl₂ (6 mmol/l) and ATP (5.6 mmol/l) (all reagents Sigma Chemical Co., Poole, Dorset, UK). Vials were incubated at 37°C for 2 h and the mixture lyophilised. Following reconstitution in 1 ml 1 N H₂SO₄ and addition of 10 ml bio-fluor (Dupont Ltd.), radioactivity was counted in a liquid scintillation counter. Total tritiated glucose radioactivity was determined by processing, in parallel with the above samples, a further 200 μ l of the

Fig. 1A–C. Plasma concentrations of glucose (**A**) and insulin (**B**) and exogenous glucose infusion rates (**C**) during glucose clamp experiments in Type 2 diabetic (Δ) and control ($\square$) subjects

original reconstituted plasma extract with 500 μ l of buffer in place of the enzyme solution. To determine $[2\text{-}^3\text{H}]$ - and $[6\text{-}^3\text{H}]$ glucose radioactivity in the tracer infusates, 100 μ l of the continuous infusate and 100 μ l of 1:10 dilution of the 20% glucose infusate (to prevent saturation of the enzyme solution by glucose) were added to patient plasma (taken prior to tracer infusion) and processed as above.

External standards of $[2\text{-}^3\text{H}]$ - and $[6\text{-}^3\text{H}]$ glucose from each infusate were also added to patient plasma and processed in parallel with each patient assay. The results were used to calculate the degree of detritiation of the two isotopes for each assay. Detritiation of $[2\text{-}^3\text{H}]$ glucose was $98.0 \pm 0.4\%$ (interassay coefficient of variation [CV] 1.2%) whereas detritiation of $[6\text{-}^3\text{H}]$ glucose was only $1.0 \pm 0.3\%$ (interassay CV 1.0%).

Insulin was determined by a radioimmunoassay with insulin antibody precipitate [13] (interassay CV 5.0%). NEFA (Wako Chemicals GmbH, Neuss, FRG; interassay CV 4.2%), B-hydroxybutyrate (Randox Laboratories Ltd., Crumlin, Antrim, UK; interassay CV 5.2%), glycerol (Randox Laboratories Ltd.; interassay CV 5.2%), pyruvate (Sigma Chemical Co., interassay CV 2.1%) and lactate (Sigma Chemical Co., interassay CV 2.5%) were all assayed by commercial kits using enzymatic methods and spectrophotometry. All CVs are given for the relevant ranges measured in this project.

Calculations

The non-steady state equations of Steele et al. [14] as modified by DeBodo et al. [15] (assuming a pool fraction value of 0.65 and an extra-cellular volume of 190 ml/kg) were used to determine rates of glucose appearance and disappearance separately for $[2\text{-}^3\text{H}]$ - and $[6\text{-}^3\text{H}]$ glucose. The EGP ($[6\text{-}^3\text{H}]$ glucose) during the 0.4 mU and 2.0 mU insulin infusion was calculated by subtraction of the exogenous glucose infusion rate required to maintain euglycaemia from the rate of glucose appearance determined with $[6\text{-}^3\text{H}]$ glucose. G/G6P cycle activity was measured as the difference between the rates of glucose appearance determined with $[2\text{-}^3\text{H}]$ - and $[6\text{-}^3\text{H}]$ glucose. This is based on the fact that $[2\text{-}^3\text{H}]$ glucose loses its label as a result of G/G6P cycle activity and that appearance rates determined with this isotope will represent appearance of cycled and non-cycled (glycogenolysis and gluconeogenesis) glucose. By contrast, during G/G6P cycling $[6\text{-}^3\text{H}]$ glucose retains its label and will estimate the appearance rate of non-cycled glucose only [16]. Results for G/G6P cycle activity in the text are expressed in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ of glucose.

Statistical analysis

All data in the text and figures are quoted as mean $\pm$ SEM. Differences in post-absorptive glucose turnover rates between the eight diabetic and eight control subjects were analysed by paired two-tailed Student's *t*-tests. Because three of the control subjects had participated in a previous study employing a different insulin infusion protocol, their results during hyperinsulinaemia were not used and the differences in glucose turnover rates between the eight diabetic and five control subjects were analysed by unpaired *t*-tests. Changes in metabolite concentrations during the clamp studies from basal were evaluated by analysis of variance. Differences in metabolite concentrations between the diabetic and control subjects were analysed by paired *t*-tests (post-absorptive) and unpaired *t*-tests (during hyperinsulinaemia).

Results

Post-absorptive plasma glucose concentrations prior to isotope (10.7 ± 1.4 vs 5.4 ± 0.0 mmol/l, $p < 0.01$) and prior to insulin (9.5 ± 0.8 vs 5.3 ± 0.0 mmol/l, $p < 0.01$) infusion were higher in the diabetic than in the control subjects. Post-absorptive serum insulin concentration (9 ± 1 vs 7 ± 1 mU/l $p = \text{NS}$) was also higher in the diabetic than in the control subjects (Fig. 1), although the difference was not statistically significant. During hyperinsulinaemia, plasma glucose concentration was allowed to fall in the diabetic subjects to 5.3 mmol/l (100 ± 19 min) and was then clamped at 5.3 ± 0.0 mmol/l (CV $3.8 \pm 0.4\%$). Mean plasma glucose concentration in the diabetic subjects during the last 30 min of the $0.4 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ clamp was 7.0 ± 1.0 mmol/l. Plasma glucose concentration in the control subjects was clamped at 5.3 ± 0.0 mmol/l (CV $3.4 \pm 0.5\%$). Plasma insulin concentrations during hyperinsulinaemia were similar in the diabetic and control subjects (0.4 mU infusion: 31 ± 4 vs 29 ± 3 mU/l; 2.0 mU infusion: 153 ± 10 vs 141 ± 12 mU/l).

The exogenous glucose infusion rates required to maintain a plasma glucose concentration of 5.3 mmol/l (Fig. 1C) during the last 30 min of the $0.4 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin infusion (3.0 ± 1.4 vs 16.8 ± 2.3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.001$) and $2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin infu-

Fig. 2. $[6\text{-}^3\text{H}]$ glucose specific activity (expressed as $\text{dpm} \times 10^3/\text{mmol}$) during glucose clamp experiments in Type 2 diabetic (Δ) and control ($\square$) subjects

Fig. 3A,B. Endogenous glucose production rates (A) and glucose disappearance rates (B) in the post-absorptive state in Type 2 diabetic ($n=8$) and control ($n=8$) subjects, and during glucose clamp experiments in Type 2 diabetic ($n=8$) and control ($n=5$) subjects. ■ represent Type 2 diabetic subjects and □, control subjects

sion (28.1 ± 2.1 vs $49 \pm 5 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.005$) were lower in the diabetic than the control subjects.

Glucose turnover rates

Analysis of variance did not show any significant change in basal glucose specific activities from -30 to 0 min in the diabetic subjects or control subjects (Fig. 2). Plasma glucose specific activity in diabetic and control subjects increased as a similar percentage of basal specific activity during 0.4 mU insulin infusion ($41 \pm 1\%$ vs $38 \pm 4\%$). During 2.0 mU insulin infusion, glucose specific activity continued to rise in diabetic subjects but decreased in the control subjects ($59 \pm 11\%$ vs $31 \pm 4\%$, $p < 0.05$). The EGP (Fig. 3A), determined with $[6\text{-}^3\text{H}]$ glucose, was greater in the diabetic subjects in the post-absorptive state (15.6 ± 1.5 vs $11.3 \pm 0.4 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.05$) and during the final 30 min of the $0.4 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin infusion (10.1 ± 1.3 vs $5.2 \pm 0.3 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.005$) when EGP is only partially suppressed. Post-absorptive EGP was strongly correlated in the diabetic subjects with fasting plasma glucose concentration ($r = 0.99$, $p < 0.001$). During the final 30 min of the $2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin infusion, when EGP is maximally suppressed, in neither diabetic nor control subjects was a negative value calculated for EGP (2.6 ± 0.5 vs $3.4 \pm 0.7 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Glucose disappearance ($[6\text{-}^3\text{H}]$ glucose) (Fig. 3B) during both the 0.4 mU (13.0 ± 0.9 vs $22.0 \pm 2.3 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.05$) and 2.0 mU (30.8 ± 2.0 vs $52.4 \pm 4.6 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.01$) insulin infusions was lower in diabetic than in control subjects. In the diabetic subjects plasma glucose disappearance during 2.0 mU insulin infusion was negatively correlated with fasting plasma glucose concentration ($r = -0.79$, $p < 0.05$) and with post-absorptive EGP ($r = -0.76$, $p < 0.05$).

Glucose cycling

Post-absorptive G/G6P cycle activity (Fig. 4) was greater in the diabetic subjects than in the control subjects (2.6 ± 0.4 vs $1.6 \pm 0.2 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.05$). During 0.4 mU insulin infusion G/G6P cycle activity was similar in the two groups (2.0 ± 0.4 vs $2.0 \pm 0.3 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) whereas during 2.0 mU insulin infusion G/G6P cycling was greater in the diabetic subjects (3.8 ± 0.7 vs $0.8 \pm 0.6 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.05$). The differences within the two groups between post-absorptive G/G6P cycle activity and cycle activity during both low- and high-dose insulin infusion did not reach statistical significance. There was no correlation between absolute G/G6P cycling and EGP in either the post-absorptive state or during hyperinsulinaemia.

Metabolites

Post-absorptive concentrations of NEFA, glycerol, pyruvate and lactate were greater in the diabetic subjects than in the control subjects (Table 2), whereas post-absorptive B-hydroxybutyrate concentration was lower in the dia-

Fig. 4. Glucose/glucose-6-phosphate cycle activity in the post-absorptive state in Type 2 diabetic ($n = 8$) and control ($n = 8$) subjects and during glucose clamp experiments in Type 2 diabetic ($n = 8$) and control ($n = 5$) subjects. ■ represent Type 2 diabetic subjects and □, control subjects

betic subjects. None of these differences however reached statistical significance.

During 0.4 mU and 2.0 mU insulin infusions, NEFA, glycerol and B-hydroxybutyrate concentrations decreased in both the diabetic and control subjects. NEFA concentrations were significantly greater in diabetic subjects during both 0.4 mU ($p < 0.05$) and 2.0 mU ($p < 0.01$) insulin infusions. Glycerol and B-hydroxybutyrate concentrations during hyperinsulinaemia were not significantly different between diabetic and control subjects. Lactate and pyruvate concentrations increased slightly during hyperinsulinaemia in the control subjects but did not alter significantly in diabetic subjects. No significant difference in lactate and pyruvate concentrations was observed between diabetic and control subjects during either the 0.4 mU or 2.0 mU insulin infusion.

Discussion

Our results confirm the presence of both hepatic and peripheral insulin resistance in Type 2 diabetes. Endogenous (hepatic) glucose production rates (EGP) in the post-absorptive state and during low-dose insulin infusion were inappropriately high for the prevailing insulin and glucose concentrations indicating hepatic insulin resistance. Peripheral insulin resistance was evident in reduced glucose disappearance rates during hyperinsulinaemia. In our diabetic subjects, and as previously reported by others [3], a strong correlation was present between post-absorptive EGP and fasting plasma glucose concentration. At the same time absolute post-absorptive glucose disappearance was increased, presumably due to the direct stimulatory effect of hyperglycaemia on muscle glucose disposal [17]. It is therefore likely that excessive glucose production by the liver has a major causal role in fasting hyperglycaemia in Type 2 diabetes.

Post-absorptive G/G6P cycle activity was greater in our diabetic subjects than in the non-diabetic control subjects.

The calculated values, expressed in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ of glucose, are very similar to those obtained by Efendic et al. [7]. In the post-absorptive state hepatic glucose flux is predominantly in the direction glucose 6-phosphate $\rightarrow$ glucose and the rate of G/G6P cycling is largely dependent on glucokinase activity. Consequently, it has been suggested that increased G/G6P cycle activity under these conditions is a response by the liver to dampen hyperglycaemia [7]. During low-dose insulin infusion we observed no difference in G/G6P cycle activity between diabetic and control subjects but during high-dose insulin infusion, cycling was over four times higher in diabetic subjects. However, when this increase in cycle activity in our diabetic subjects during high-dose insulin was compared to the decrease in glucose disappearance, it represented only 14% of the deficit. It thus seems likely that increased G/G6P cycle activity does indeed contribute to insulin resistance in Type 2 diabetes by reducing net hepatic glucose uptake and increasing overall hepatic glucose output when hepatic glucose flux is predominantly reversed from glucose output to uptake. However, under these conditions the contribution of G/G6P cycle activity to insulin resistance in Type 2 diabetes is relatively small compared to the impaired peripheral tissue glucose utilisation. This conclusion, based on studies in fasted patients under hyperinsulinaemic euglycaemic conditions, is similar to the findings of Butler and Rizza [18] in patients studied post-prandially. Whilst they found that overall hepatic glucose cycling (G/G6P cycling and concurrent glycogen synthesis/glycogenolysis) was increased in Type 2 diabetes during the first 2 h after food ingestion, hyperglycaemia was mostly due to excessive hepatic glucose release and failure of appropriate extra-hepatic glucose uptake.

What mechanisms could account for greater G/G6P cycle activity in Type 2 diabetes during high-dose insulin infusion? Efendic et al. [7] have suggested that, in Type 2 diabetes, activity of the enzyme glucose 6-phosphatase might be inadequately suppressed under conditions where hepatic glucose uptake is predominant. The K_m of glucose 6-phosphatase (2–4 mmol/l) is, however, far above the usual physiological hepatic concentration of its substrate glucose 6-phosphate (0.1 mmol/l) [19], implying that under such conditions G/G6P cycle activity is instead largely dependent on the supply of glucose 6-phosphate. The hepatic glucose 6-phosphate concentration will remain high during hyperinsulinaemia if EGP is inadequately suppressed. NEFA can increase EGP by enhancing gluconeogenesis [20] and decreasing NEFA availability in Type 2 diabetes improves the suppression of EGP by insulin [21]. However, during 2.0 mU insulin infusion in the present study, although NEFA levels were higher in the diabetic subjects, they were considerably reduced at approximately 25% of the post-absorptive levels. Glucagon also stimulates EGP [22] and it is recognised that many patients with Type 2 diabetes are hyperglucagonaemic [23]. Glucagon infusion has been shown to increase G/G6P cycling in dogs [24] and man [25] and hepatic glucose cycling in Type 2 diabetes during the first 2 h after a meal correlates with the plasma glucagon concentration [18].

Table 2. Metabolite concentrations (mean $\pm$ SEM) in the post-absorptive state and during hyperinsulinaemic glucose clamp studies in Type 2 diabetic and control subjects

	NEFA (mmol/l)	Glycerol (μ mol/l)	B Hydroxybutyrate (mmol/l)	Pyruvate (mmol/l)	Lactate (mmol/l)
<i>Post-absorptive</i>					
Diabetic subjects ($n = 8$)	0.72 ± 0.06	67.7 ± 10.2	0.07 ± 0.02	0.09 ± 0.02	0.85 ± 0.20
Control subjects ($n = 8$)	0.54 ± 0.03	55.9 ± 6.4	0.16 ± 0.03	0.07 ± 0.02	0.62 ± 0.15
<i>0.4 mU Insulin</i>					
Diabetic subjects ($n = 8$)	0.39 ± 0.06^a	41.5 ± 6.4	0.03 ± 0.00	0.09 ± 0.02	0.79 ± 0.23
Control subjects ($n = 5$)	0.16 ± 0.02	27.3 ± 5.1	0.05 ± 0.01	0.08 ± 0.01	0.63 ± 0.07
<i>2.0 mU Insulin</i>					
Diabetic subjects ($n = 8$)	0.19 ± 0.04^a	33.3 ± 6.5	0.00 ± 0.00	0.10 ± 0.01	0.91 ± 0.16
Control subjects ($n = 5$)	0.03 ± 0.00	19.8 ± 5.7	0.01 ± 0.00	0.09 ± 0.00	0.86 ± 0.11

^a $p < 0.05$.

NEFA, Non-esterified fatty acids

Finally, in the present study insulin action was assessed by the euglycaemic hyperinsulinaemic clamp employing an exogenous glucose infusion labelled so as to prevent decline in plasma glucose specific activity because previous studies [8, 26] have shown that it successfully eliminates the "negative" glucose production rates commonly seen during high-dose insulin infusions in conventional clamp studies. Using this technique, a rise in glucose specific activity is often observed [8]. However, although glucose specific activity was similarly steady in the diabetic and control subjects in the post-absorptive state, this rose to a greater extent in the diabetic subjects during hyperinsulinaemia. This was mainly because baseline plasma glucose concentration in the diabetic subjects was allowed to decline to 5.3 mmol/l, so that glucose disappearance in both groups would be determined at similar levels of glycaemia. As the exogenous glucose infusion in the diabetic subjects was labelled to account for the much higher post-absorptive glucose concentration, maintaining the lowered concentration of 5.3 mmol/l inevitably resulted in a greater rise in plasma glucose specific activity. It is unlikely that this increase in glucose specific activity significantly affected our calculations of G/G6P cycle activity during hyperinsulinaemia because it is probable that any error would apply similarly to both [2-³H]- and [6-³H]glucose determined glucose turnover rates.

In conclusion, the present study shows that significant G/G6P cycle activity occurs in Type 2 diabetes and is considerably increased under conditions where EGP is maximally suppressed and glucose turnover is high. However, even under these latter conditions G/G6P cycle activity is relatively small compared to the deficit in total glucose disappearance. Consequently, it seems unlikely that G/G6P cycle activity contributes greatly to insulin resistance in Type 2 diabetes, but rather that insulin resistance is mainly due to excessive EGP and reduced peripheral glucose disposal.

Acknowledgements. The authors wish to thank the nursing staff of the Sir George E. Clark Metabolic Unit for help with the studies and Dr. C. C. Patterson, Department of Epidemiology and Public Health Medicine, Queen's University of Belfast, for help in the analysis of data.

This study was supported by a British Diabetic Association Research Grant. DPR is the recipient of a Royal Victoria Hospital Research Fellowship.

References

1. Reaven GM, Chen Y-DI, Donner CC, Frazee E, Hollenbeck CB (1985) How insulin resistant are patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 61: 32–36
2. Firth R, Bell PM, Rizza RA (1987) Insulin action in non-insulin-dependent diabetes mellitus: the relationship between hepatic and extrahepatic insulin resistance and obesity. *Metabolism* 36: 1091–1095
3. Campbell PJ, Mandarino LJ, Gerich JE (1988) Quantification of the relative impairment in actions of insulin on hepatic glucose production and peripheral glucose uptake in non-insulin-dependent diabetes mellitus. *Metabolism* 37: 15–21
4. Butler PC, Kryshak EJ, Schwenk WF, Haymond MW, Rizza RA (1990) Hepatic and extrahepatic responses to insulin in NIDDM and nondiabetic humans. Assessment in absence of artifact introduced by tritiated nonglucose contaminants. *Diabetes* 39: 217–225
5. McGarry JD, Kuvoajima M, Newgard CB, Foster DW (1987) From dietary glucose to liver glycogen: the full circle round. *Annu Rev Nutr* 7: 51–73
6. Katz J, Rognstad R (1976) Futile cycles in the metabolism of glucose. *Curr Top Cell Regul* 10: 237–289
7. Efendic S, Karlander S, Vranic M (1988) Mild type II diabetes markedly increases glucose cycling in the postabsorptive state and during glucose infusion irrespective of obesity. *J Clin Invest* 81: 1953–1961
8. Neely RDG, Rooney DP, Atkinson AB, Sheridan B, Ennis CN, Trimble ER, Bell PM (1990) Underestimation of glucose turnover determined using [6-³H]glucose in non-steady state. The role of a tritiated tracer impurity. *Diabetologia* 33: 681–687
9. Rooney DP, Neely RDG, Ennis CN, Bell NP, Sheridan B, Atkinson AB, Trimble ER, Bell PM (1992) Insulin action and hepatic glucose cycling in essential hypertension. *Metabolism* 41: 317–324
10. Levy JC, Brown G, Matthews DR, Turner RC (1989) Hepatic glucose output in humans measured with labeled glucose to reduce negative errors. *Am J Physiol* 257: E531–E540
11. Somogyi M (1945) Determination of blood sugar. *J Biol Chem* 160: 69–73
12. Issekutz B (1977) Studies on hepatic glucose cycles in normal and methylprednisolone treated dogs. *Metabolism* 26: 157–170
13. Hales CN, Randle PJ (1963) Immunoassay of insulin with insulin antibody precipitate. *Biochem J* 88: 137–146

14. Steele R, Wall JS, De Bodo RC, Altszuler N (1956) Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187: 15–25
15. De Bodo RC, Steele R, Altszuler N, Dunn A, Bishop JS (1963) On the hormonal regulation of carbohydrate metabolism: studies with C^{14} glucose. *Rec Prog Horm Res* 19: 445–448
16. Karlander S, Roovete A, Vranic M, Efendic S (1986) Glucose and fructose 6-phosphate cycle in humans. *Am J Physiol* 251: E530–E536
17. Capaldo B, Santoro D, Riccardi G, Perrotti N, Sacca L (1987) Direct evidence for a stimulatory effect of hyperglycaemia per se on peripheral glucose disposal in type 2 diabetes. *J Clin Invest* 77: 1285–1290
18. Butler PC, Rizza RA (1991) Contribution to postprandial hyperglycaemia and effect on initial splanchnic glucose clearance of hepatic glucose cycling in glucose-intolerant or NIDDM patients. *Diabetes* 40: 73–81
19. Nordlie R (1974) Metabolic regulation by multifunctional glucose-6-phosphatase. *Curr Top Cell Regul* 8: 33–117
20. Williamson JR, Kreisberg RA, Felts PW (1966) Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. *Proc Natl Acad Sci USA* 56: 247–254
21. Saloranta C, Franssila-Kallunki A, Ekstrand A, Taskinen M-R, Groop L (1991) Modulation of hepatic glucose production by non-esterified fatty acids in type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 34: 409–415
22. Liljenquist JE, Mueller GL, Cherrington AD et al. (1977) Evidence for an important role of glucagon in the regulation of hepatic glucose production in normal man. *J Clin Invest* 59: 369–374
23. Raskin P, Unger RH (1978) Hyperglucagonaemia and its suppression, importance in the metabolic control of diabetes. *N Engl J Med* 299: 433–436
24. Lickley H, Kemmer F, El-Tayeb K, Vranic M (1987) Importance of glucagon in the control of futile cycling as studied in alloxan-diabetic dogs. *Diabetologia* 30: 175–182
25. Miyoshi H, Shulman G, Peters E, Wolfe M, Elahi D, Wolfe R (1988) Hormonal control of substrate cycling in humans. *J Clin Invest* 81: 1545–1555
26. Finegood DT, Bergman RN, Vranic M (1987) Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes* 36: 914–924

Received: 15 June 1992
and in revised form: 21 September 1992

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