No glucotoxicity after 53 hours of 6.0 mmol/l hyperglycaemia in normal man

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Summary. In vitro and in vivo studies have suggested that metabolic deterioration can be induced by hyperglycaemia *per se*. The effect of 53 h of 2.2 mg glucose·kg ideal body weight⁻¹·min⁻¹ was examined in four normal male subjects. This produced overnight hyperglycaemia of 6.0 mmol/l on the two nights of the study compared with 4.7 mmol/l on the control night ($p < 0.05$). In response there was a sustained, two-fold increase in basal plasma insulin ($p < 0.005$) and C-peptide ($p < 0.05$) levels. After two days of hyperglycaemia an increased Beta-cell response was demonstrated in response to an additional glucose infusion stimulus (estimated Beta-cell function median of 84% on the control day to 100% after two days glucose infusion). Plasma insulin and C-peptide responses to a 10.0 mmol/l hyperglycaemic clamp

increased over the two days of the study (insulin from median 48 mU/l to 73 mU/l and C-peptide from median 2.0 pmol/ml to 2.6 pmol/ml). Glucose tolerance to the additional glucose infusion stimulus improved, suggesting that the increased insulin response during hyperglycaemia was enhancing peripheral glucose uptake. The calculated peripheral insulin sensitivity was unchanged during the hyperglycaemic clamp. Thus, in response to the two days of basal hyperglycaemia, both the basal and stimulated Beta-cell responses were enhanced and there was no evidence for 'glucose toxicity' to the Beta-cells.

Key words: Insulin, glucose, insulin resistance, man, glucotoxicity.

Four decades ago Lukens and Dohan [1] proposed that hyperglycaemia *per se* could play a pathogenic role in diabetes. Since then various animal models have been used to demonstrate that glucose may be directly toxic to the Beta cell. Leahy et al. [2] found that 48 to 96 h infusion of glucose in Sprague-Dawley rats caused a loss of glucose-stimulated insulin secretion, and in normal man, short-term (2–12 h) studies of glucose infusion [3–5] caused Beta-cell insensitivity to hyperglycaemia with decreased first-phase response to intravenous glucose. Type 2 (non-insulin-dependent) diabetic subjects with a fasting plasma glucose > 6.4 mmol/l have a markedly impaired first-phase response to intravenous glucose [6] and a decreased glucose potentiation of non-glucose stimuli [7]. Conversely, Beta-cell responsiveness to glucose appears to be restored towards normal by eliminating pre-existing hyperglycaemia by diet [8] sulphonylureas [9] or insulin [10, 11]. There is thus evidence, both in animal and human studies, that hyperglycaemia may attenuate the Beta-cell response to glucose *per se*. In addition, it may impair insulin-mediated glucose transport and may initiate a cycle of metabolic deterioration. This has been called the 'hyperglycaemia cycle' [12].

Previous studies of the effects of hyperglycaemia on Beta-cell function in normal man have been for periods of up to 20 h and have not included studies of insulin sensitivity. The aim of our study was to assess the effect of 53 h of mild hyperglycaemia on Beta-cell function, hepatic glucose production and peripheral glucose uptake.

Subjects and methods

Four subjects were studied (Table 1) and, because of the length and complexity of the study, all were male medical staff who were well-informed and gave consent. None of the subjects had a family history of diabetes. For infusion purposes ideal body weight (IBW) was calculated for medium frame subjects from the Metropolitan Life Assurance Co. (1959). Body mass index was calculated as weight in kg/(height in metres)². Each subject was studied on two occasions: a control day and a prolonged study during which they were given a continuous glucose infusion of 2.2 mg·kg IBW⁻¹·min⁻¹ glucose for a period of 53 h (Fig. 1). This approximates the basal hepatic glucose output (HGO) in normal man [13] and produces an 85% reduction in HGO with no effect on peripheral glucose utilization when given over a 45-min period in normal man [14]. The two studies were separated by a minimum period of one week and performed in random order. The study was approved by the Central Oxford Research Ethics Committee.

Table 1. Clinical details of the four subjects studied

Subject	1	2	3	4	Median
Age (years)	33	39	48	41	40
Weight (kg)	74	74	74	61	74
IBW (%)	93	100	102	80	96
BMI	21.0	22.1	23.2	18.2	21.5
Calories	2500	2396	1954	2200	2298

Calories refers to the average daily calories assessed from three dietary record charts recorded before the study. Body mass index (BMI) was calculated as weight in kg/(height in m)². Ideal body weight (IBW) was taken from the Metropolitan Life Assurance Co tables (1959)

Table 2. Glucose, Insulin and C-peptide results

	Glucose (mmol/l)	Insulin (mU/l)	C-peptide (pmol/ml)
Control night (24.00–06.00 hours)	4.7	6.4	0.4
Night 1 (24.00–06.00 hours)	6.3 ^b	11.6 ^b	0.9 ^a
Night 2 (24.00–06.00 hours)	6.2 ^a	11.7 ^b	1.1 ^a
Control CIG	9.7	16.8	1.1
Day 2 CIG	8.5 ^b	23.4	1.5
Day 3 CIG	7.9 ^b	33.4 ^a	1.7 ^a
Control clamp	9.8	48.3	2.0
Day 2 clamp	10.3	53.5	2.0
Day 3 clamp	10.2	73.3 ^a	2.6 ^a

^a*p* < 0.05 compared with control day; ^b*p* < 0.005 compared with control day

Median values for glucose, insulin and C-peptide for overnight studies from 24.00 hours to 06.00 hours; continuous glucose infusion (CIG) values are the achieved values for the last 15 min of the 90 min infusion; clamp values shown are for the last 30 min of the hyperglycaemic clamp.

Statistical analysis was performed on the individual data points using MANOVA on an SPSS program using the control values as comparison

Diet

Detailed dietary records of each subject's normal daily consumption of carbohydrate, fat, protein and overall calories were taken for 2–3 days and analysed by a dietitian (Table 1). On each study day subjects were fasted overnight until the end of the hyperglycaemic clamp. A meal was served at 13.00 hours and 18.00 hours on each day, and the normal total energy intake for each subject was maintained throughout the study.

Control day

On the control day plasma samples for glucose, insulin and C-peptide assay were taken at 2 h intervals for 10 h prior to 23.00 hours. An evening meal was given at 18.00 hours. The subject was then fasted until the end of the control study. At 23.00 hours the patient was admitted to hospital. A double lumen 21 gauge Teflon catheter (Venflon 2, Viggo, Helsingborg, Sweden) was placed into a distal forearm vein under local anaesthetic and warmed to 'arterialise' the blood and enhance flow. Heparin was infused through the outer lumen at 1/100 of the blood extraction rate. Blood samples were taken continuously [15] collecting integrated samples over each 15-min period overnight for glucose, insulin, C-peptide and growth hormone measurements. At 08.00 hours the next morning basal plasma samples were taken. HGO was assessed using a 90 min primed continuous infusion of 3-³H glucose (duPont New England Nuclear, Stevenage, UK) [16]. Glucose turnover was not performed in the one subject < 35 years for ethical reasons. At times 75, 80, 85 and 90 min of the infusion 3.5 ml blood samples were taken. At 90 min a continuous in-

fusion of glucose (CIG) at 5 mg·kg IBW⁻¹·min⁻¹ [17] was commenced. At times 95, 100, 110, 120, 130, 140, 150, 160, 165, 170, 175 and 180 min 3.5 ml samples for glucose, insulin and C-peptide assay were taken. The CIG was followed by a standard 2 h 10 mmol/l glucose hyperglycaemic clamp attained by variable 20% glucose infusion [18]. At times 15, 30, 45, 60, 75, 90, 105, 110, 115 and 120 min of the clamp 3.5 ml samples for glucose, insulin and C-peptide assay were taken. Control days and the study days were in random order.

The study days

For the study period the subject was admitted to hospital at 08.00 hours after an overnight fast. A 21 gauge teflon catheter was inserted into a distal warmed forearm vein to obtain integrated blood samples as described above. A second 21 gauge teflon catheter was inserted into a antecubital vein of the other arm for the continuous infusion of 10% glucose. HGO was assessed as on the control day. A 10% continuous glucose infusion of 2.2 mg·kg IBW⁻¹·min⁻¹ was commenced at 09.30 hours using a volumetric infusion pump (IMED, Milton Trading Estate, Abingdon, UK) and this infusion continued until the end of the study. Samples were taken every 2 h until 23.00 hours followed by overnight 15 min integrated samples.

On the second day of the study at 09.30 hours a 90 min basal assessment of hepatic glucose output using 3-³H glucose was followed by a 90 min CIG. This was followed by a 2 h 10 mmol/l hyperglycaemic clamp. The basal glucose infusion was then continued and the subject given small meals to make up the usual daily intake of calories, protein and fat. A further overnight study, identical to the control night and to the first night of the study was followed on the third day by repeating the protocol of the second day and the study ended after the hyperglycaemic clamp. The energy provided during the study was isocaloric to the subjects' average calorie consumption and meals were calculated to provide the same dietary composition as their normal diet (approximately 50% carbohydrate, 30% fat and 20% protein). Although subjects were supine while sleeping and during the clamp studies, they were ambulant with an infusion pump at other times. The calories provided by the constant glucose infusion, the glucose tolerance infusion test and the hyperglycaemic clamp were taken into account.

Assays

Insulin and C-peptide levels were assayed by charcoal-phase separation radioimmunoassay [19] with an intra-assay coefficient of variation (CV) of 9.8 at 6.9 mmol/l (insulin) and 9.2% at 0.72 nmol/l (C-peptide) and a between-assay coefficient of variation of 17% and 8%

Fig. 1. The study protocol. Upper panel: control day. Lower panel: the 53 h protocol

Fig. 2. Results from the overnight studies: Upper panel; median glucose concentrations, centre panel; median insulin concentrations, lower panel; median C-peptide concentrations. Δ = control day, $\bullet$ = night 1, + = night 2

Fig. 3. Histogram of the Beta-cell function for the four subjects assessed after an additional glucose infusion using the CIGMA model [17]. Control day = open bar; day 2 = cross-hatched; day 3 = solid

respectively. Glucose was measured by a glucose-oxidase method with a between-assay coefficient of variation of 3%.

Beta-cell function at the end of the $5 \text{ mg} \cdot \text{kg IBW}^{-1} \cdot \text{min}^{-1}$ glucose infusion was assessed by reference to a mathematical model of glucose/insulin relationships—continuous infusion of glucose with model assessment (CIGMA) using the mean of the 50, 55 and 60 min values and expressed as a percentage of a reference normal population [17]. Using this model, insulin resistance (assessed by the glucose lowering

Fig. 4. The insulin (upper panel) and C-peptide (lower panel) concentrations at the end of the hyperglycaemic clamps for the individual subjects on the control day, day 2 and day 3. Subjects 1–4 indicated by symbols +, O, +, Δ respectively

effect of insulin) cannot be interpreted in the presence of additional extraneous glucose. Beta-cell function, assessed by the insulin response to hyperglycaemia, can still be measured. Insulin secretion was assessed from the hyperglycaemic clamp by the mean of the last four values (last 15 min of clamp). Insulin resistance was assessed by the glucose infusion rate (M) during the last 40 min hyperglycaemic clamp divided by the corresponding insulin concentration (M/I).

Statistical analysis

The results were analysed using MANOVA on a Statistical Package for the Social Sciences Programme (Norussis and SPSS 1988) [20]; time was used as a separate variable.

Results

Basal and overnight concentrations

The median basal concentration of glucose from 06.00 hours to 08.00 hours on the control day was 4.7 (4.6–4.8) and increased to 6.0 (5.8–6.1) and 6.1 (5.7–6.2) mmol/l on the second and third day of the study respectively. The corresponding median basal concentration of insulin increased from 4.9 (3.2–6.2) to 12.8 (6.2–13.6), and 11.2 (7.3–12.2) mU/l respectively, and the median C-peptide concentration increased from 0.32 (0.1–0.34) to 0.88 (0.45–1.1) and 0.81 (0.59–1.0) pmol/ml respectively. The overnight (24.00 to 06.00 hours) profiles of glucose, insulin and C-peptide are shown in Table 2 and Figure 2.

Model Assessment of Beta-cell function

Beta-cell function assessed by CIGMA [17] gave a median of 84% on the control day, 87% on the second day and 100% on the third day of the study (Fig. 3). In all subjects

Table 3. Insulin sensitivity

Subjects	1	2	3	4	Median
Control	7.0	8.8	7.2	5.8	7.1
Day 2	5.9	9.8	11.1	3.9	7.8
Day 3	6.3	6.7	4.9	3.3	5.6

The insulin sensitivity (M/I) during hyperglycaemic clamp is assessed from the glucose infused (mg total) relative to the mean plasma insulin concentration (mU/l) for the last 40 min of the clamp

Table 4. Basal hepatic glucose output

Subject	2	3	4	Median
Control day	8.7	9.0	8.7	8.7
Day 1	10.8	10.2	9.2	10.2
Day 2	2.6	3.4	-1.3	2.6
Day 3	-2.7	4.7	-1.5	-1.5

Basal hepatic glucose output was determined isotopically using $3\text{-}^3\text{H}$ glucose and is expressed in $\mu\text{mol}\cdot\text{kg IBW}^{-1}\cdot\text{min}^{-1}$. $p < 0.01$ (Anovar control vs Day 2 and 3)

No measurements were performed in subject 1

the Beta-cell function on day 3 was higher than either the control day or day 2.

Hyperglycaemic clamps

Plasma insulin and C-peptide concentrations during the last 30 min of the hyperglycaemic clamps are shown in Figure 4. There was a clear rise in the plasma insulin response to hyperglycaemia during the 53 h glucose infusion, with no overlap between the insulin concentration on the second day of the study and the control day ($p < 0.05$). The glucose clamp data showed no significant change in insulin sensitivity as estimated from glucose uptake per mU insulin (M/I) (Table 3).

Glucose turnover

The glucose infusion caused a suppression of hepatic glucose turnover, from a median of $10.2\ \mu\text{mol}\cdot\text{kg IBW}^{-1}\cdot\text{min}^{-1}$ to 2.6 and -1.5 on days 2 and 3 respectively (Table 4). For comparison the infusion rate was $2.2\ \text{mg}\cdot\text{kg IBW}^{-1}\cdot\text{min}^{-1}$, that is $12.2\ \mu\text{g}\cdot\text{kg IBW}^{-1}\cdot\text{min}^{-1}$. The occurrence of negative turnover values may be due to the use of non-high performance liquid chromatography-purified tracer.

Discussion

The basal infusion of glucose, equivalent to the normal hepatic glucose output, induced overnight hyperglycaemia on both the first night, mean 6.4 mmol/l, and the second night, 6.3 mmol/l, compared with 4.8 mmol/l on the control night. In response to the basal hyperglycaemia there was a sustained, two-fold increase in basal plasma insulin and C-peptide levels with no evidence of Beta-cell fatigue. Indeed, in response to the additional constant infusion of glucose stimulus used to assess Beta-cell function, the insulin and C-peptide responses increased over the two days of the investigation, even though the plasma glucose levels

attained at the end of the additional glucose infusion were lower than on the control day. This increased Beta-cell response was also apparent from the increased plasma insulin and C-peptide concentration during the standard 10 mmol/l hyperglycaemic clamp. The improved glucose tolerance during CIG suggests that hyperglycaemia and the increased insulin response enhanced peripheral glucose uptake (particularly as basal glucose delivery was artificially maintained by continuation of the basal, exogenous glucose infusion, whereas normally the basal hepatic glucose output is suppressed during the test). The glucose infusion required during the hyperglycaemic clamp to achieve 10 mmol/l indicated peripheral insulin sensitivity was unchanged. Thus, in response to two days of basal hyperglycaemia, both the basal and stimulated Beta-cell responses were enhanced and there was no evidence for 'glucose toxicity' to the Beta-cells.

In vitro studies of the response to hyperglycaemia of perfused islets [21], batch-incubated islets [22] and the perfused pancreas [23] up to 24 h showed that after the initial first-phase release, the second-phase insulin secretion increases over 2–3 h of constant glucose stimulation. This time-dependent increase in insulin release during constant stimulation reflects the ability of glucose and other secretagogues to amplify their own signal and is referred to as 'time-dependent potentiation' or 'priming' [24]. In both animals [25] and normal man [26, 27] a prolonged glucose infusion resulted in sustained high blood insulin levels, as was also found over a period of 53 h in the present study. The level of glycaemia achieved during the present study is similar to that found in experimental insulin resistance [28] and the results would be in accord with the increased insulin responses of obesity [29] being in part secondary to slightly increased basal plasma glucose levels. It is pertinent to observe that basal hyperglycaemia can be achieved by a glucose infusion which is no greater in quantity than the normal caloric intake. This is presumably related to the infusion causing an artificial glucose "output" which is only 10% higher than the subjects' measured basal HGO. Thus, overnight normoglycaemia seems to be critically dependent on control of hepatic glucose production.

Porte and Pupo [26] studied two rates of glucose infusion of 100 mg/min and 300 mg/min for 20 h and showed a sustained hyperinsulinaemic response to the glycaemic stress. In response to an intravenous glucose bolus tolerance test, the second phase of insulin secretion was greater in proportion to the degree of basal hyperglycaemia, although the test did not allow comparison of responses at identical glucose concentrations. Ward and colleagues [27] showed that 20 h infusion of glucose which raised the plasma glucose from 95 to 130 mg/dl (5.3 to 7.2 mmol/l) caused a consistent increase in both the acute insulin and glucagon response to intravenous arginine.

The present study has examined the Beta-cell response to 6.0 mmol/l glucose, and has demonstrated an enhanced rather than the depleted Beta-cell response found in vitro studies. It is not possible to determine whether there is a species difference, or a difference between insulin secretion in vivo and that assessed subsequently in in vitro studies. The concept of glucose-mediated pancreatic

Beta-cell toxicity has not been borne out by the long-term exposure of isolated islets to high glucose concentrations [30–32]. The experimental data for the glucose toxicity concept comes from in vitro studies of the acute Beta-cell responses to glucose or arginine stimuli following hyperglycaemia induced by in vivo glucose infusion in rats or in two Beta-cell deficient models, the neonatal streptozotocin model and the partial pancreatectomy model [25]. These studies have shown high basal insulin secretion which could be interpreted as a continued, appropriate 'primed' physiological response, even though the responses to further hyperglycaemia or arginine stimulus were abnormal. In Type 2 (non-insulin-dependent) diabetic man it has been shown that prolonged basal hyperglycaemia inhibits the first-phase of insulin secretion [10] but the mechanisms governing the first phase response are quite complex [24, 26] and difficult to interpret. It may not be correct to term an abnormal acute response as 'glucose toxicity' when basal responses continue to be appropriate.

The present study does not determine what the Beta-cell response would be to more prolonged or greater hyperglycaemia. The occurrence of the 'honeymoon' phase after initial therapy in severely hyperglycaemic, newly presenting Type 1 (insulin-dependent) diabetic patients indicates that marked hyperglycaemia can be detrimental to Beta-cell function [33], but the fasting plasma glucose probably has to be above 12 mmol/l before this occurs [34]. In vivo infusion of high glucose concentrations in cats [1] and dogs [35] can deleteriously affect Beta-cell function. Hyperglycaemia > 10 mmol/l for 3 months in rats can induce fibrosis and islet disorganisation [36]. However, the present study suggests that modest hyperglycaemia in man probably potentiates Beta-cell secretion rather than being deleterious, at least over a 53 h period.

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