Effects of Glycemic Control on Protective Responses Against Hypoglycemia in Type 2 Diabetes

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OBJECTIVE — To determine the effects of glycemic control on the counterregulatory responses to hypoglycemia in type 2 diabetes.

RESEARCH DESIGN AND METHODS — Seven poorly controlled type 2 diabetes patients (mean HbA_{1c}, 11.3 ± 1.1%) were studied by stepped hyperinsulinemic hypoglycemic clamp (nadir, 2.4 mmol/l) before and after improving glycemic control with insulin treatment. Counterregulatory hormones, symptoms, and four-choice reaction time were measured at each glucose plateau.

RESULTS — In patients with poorly controlled type 2 diabetes, counterregulatory hormone responses began at higher plasma glucose levels than did those in healthy subjects (epinephrine, 4.4 ± 0.2 vs. 3.7 ± 0.2 mmol/l, $P = 0.011$). After significant improvement in glycemic control (mean HbA_{1c}, 8.1 ± 0.9%, $P < 0.001$) was achieved without severe hypoglycemia, hormonal responses started at much lower plasma glucose levels (e.g., epinephrine, 3.5 ± 0.3 mmol/l, $P = 0.005$) and were significantly reduced in magnitude (e.g., area under epinephrine response curve, 306 ± 93 vs. 690 ± 107 nmol · min^{-1} · l^{-1}, $P = 0.012$). This was accompanied by a change in the plasma glucose threshold at which hypoglycemic symptoms first developed from 3.6 ± 0.2 to 3.0 ± 0.2 mmol/l ($P = 0.019$). In contrast, the plasma glucose threshold at which four-choice reaction time deteriorated did not change significantly (3.1 ± 0.1 vs. 2.9 ± 0.1 mmol/l, $P = 0.125$).

CONCLUSIONS — Counterregulatory responses begin at normoglycemia in poorly controlled type 2 diabetes. Improving glycemic control with insulin therapy normalizes hormonal responses but lowers the plasma glucose levels at which hypoglycemic symptoms develop to levels associated with impairment of four-choice reaction time, a marker of cognitive function. This process potentially increases the risk of severe hypoglycemia, but to a lesser extent than occurs in type 1 disease.

The symptomatic and hormonal responses to hypoglycemia have been extensively studied in healthy subjects and in patients with type 1 diabetes. It has been shown that the magnitude of such responses is influenced by previous hypoglycemic experience, and that even one or two episodes of hypoglycemia can blunt subsequent responses for several days (1–7). After repeated episodes, symptom responses are delayed, and cognitive dysfunction may occur before or in the absence of symptoms, resulting in the potentially dangerous phenomenon of hypoglycemia unawareness (8–12).

From the time they begin treatment with sulfonylureas or insulin to lower blood glucose, patients with type 2 diabetes are also at risk of developing significant hypoglycemia (13). Being typically older, type 2 patients may be at greater risk of developing asymptomatic hypoglycemia, because normal aging is associated with blunting of symptoms and increased susceptibility to cognitive impairment (14,15). This situation may be particularly problematic when insulin is used to maintain good glycemic control in patients with long-standing type 2 diabetes, who are considered on clinical grounds to require insulin treatment and who may be more akin to type 1 patients. There have been few reported studies of the responses to hypoglycemia in type 2 diabetes (16–20), and none of these studies addressed the effects of previous glycemic experience.

We therefore studied counterregulatory hormone responses, symptoms, and four-choice reaction time (as a marker of cognitive function [21,22]) during controlled experimental hypoglycemia in a group of seven poorly controlled type 2 patients, before and after improving glycemic control with insulin treatment, using a stepped hypoglycemic clamp protocol to allow determination of the plasma glucose thresholds at which these responses begin.

RESEARCH DESIGN AND METHODS

Subjects

Five male and two female C-peptide–positive patients with type 2 diabetes, with a mean age of 56 ± 4 years (SD) and a mean duration of diabetes of 12 ± 3 years, were recruited from clinics at Guy's and King's College Hospitals in London. All patients had inadequate glycemic control when receiving maximum treatment with a sulfonylurea drug and metformin (mean HbA_{1c}, 11.3 ± 1.1%). They were defined as type 2 on the basis of their age of onset, prolonged disease duration without the need for insulin therapy, absence of any history of diabetic ketoacidosis, and C-peptide levels during euglycemia (achieved by overnight low-dose insulin infusion) in the nondiabetic range (Table 1). With one exception (BMI, 29.2 kg/m^2), patients were not obese

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Table 1—*Patient demographic data at time of recruitment*

Sex	Age (years)	Diabetes duration (years)	Weight (kg)	BMI (kg/m²)	HbA_{1c} (%)	C-peptide (ng/ml)	Drug treatment (daily dose)
M	56	16	58.2	20.1	13.5	0.13	Metformin (1,000 mg) Gliclazide (320 mg)
M	60	15	76.5	25.0	9.6	0.36	Metformin (1,000 mg) Glibenclamide (20 mg)
M	56	9	93.6	29.2	11.1	0.56	Metformin (1,700 mg) Glipizide (20 mg)
M	55	12	78.3	25.9	11.2	1.33	Gliclazide (320 mg)
M	54	7	88.5	25.8	11.4	0.43	Metformin (2,000 mg) Glibenclamide (15 mg)
F	49	14	73.9	NA	10.9	0.24	Metformin (1,500 mg)
F	59	9	68.7	24.3	11.5	1.21	Metformin (1,700 mg) Glibenclamide (20 mg)

Drug treatment and doses listed refer to drugs taken immediately before commencement of insulin treatment. No patients had been treated with insulin previously. C-peptide concentrations quoted are fasting values obtained on the morning of the first clamp study. *NA, not available.

(BMI, <26 kg/m²). Two patients had background retinopathy at recruitment, but none had evidence of other complications.

Results were compared with those from seven healthy nondiabetic men aged 65 ± 3 years who were studied according to the same protocol. None of the seven were obese (BMI, 23–26 kg/m²), and all had normal HbA_{1c} measurements and no family history of diabetes. Data on these subjects have been published previously (14).

All subjects were screened by history, physical examination, and electrocardiography to exclude ischemic heart disease, epilepsy, and hypertension. None were taking any medication other than oral hypoglycemic agents.

Hypoglycemic clamp protocol

Patients were admitted the night before the first study, having discontinued oral hypoglycemic agents the previous morning. Normoglycemia was maintained by administration of a variable infusion of soluble human insulin (Human Actrapid, Novo Nordisk, Crawley, U.K.) adjusted on the basis of half-hourly glucose measurements.

The following morning, a retrograde cannula was placed in a vein in the nondominant hand, which was kept at 55°C to arterialize venous blood (23). Not less than 30 min after placing this second cannula, arterialized blood was drawn for measurement of glucose and baseline counterregulatory hormones. A primed infusion of soluble human insulin in normal saline containing 4% autologous blood was then started and maintained at a rate of 1.5 mU · kg^{-1} · min^{-1} for the duration of the study. Plasma glucose, measured at the bedside every 5 min, was maintained at 6 mmol/l for 40 min, then reduced in successive steps to 5, 3.8, 3.4, 2.8, and 2.4 mmol/l, before being restored to 6 mmol/l. Each step lasted 40 min, except for the nadir, which lasted 20 min. At the midpoint and end of each step, arterialized blood was collected for later hormone measurement, four-choice reaction time was measured as described previously (14), and symptoms of hypoglycemia were recorded, using a standardized questionnaire in which 10 symptoms (sweating, anxiety, tremor, palpitations, feeling hot, difficulty in speaking, confusion, dizziness, irritability, and drowsiness) were ranked individually from 1 (absent) to 7 (very severe).

For the nondiabetic subjects, who had been studied according to a similar protocol but were fasting when admitted on the day of the hypoglycemic clamp study, the initial 6 mmol/l step was omitted and baseline hormone measurements were made at 5 mmol/l.

Clinical intervention

After the initial clamp, all patients were started on twice-daily premixed insulin (Human Mixtard 30 ge, Novo Nordisk) in place of previous medication and were asked to maintain blood glucose monitoring at home, four to six times daily. Insulin dose was adjusted on the basis of these readings, after weekly consultation, with the aim of achieving consistent fasting and preprandial blood glucose of 4–9 mmol/l but avoiding symptomatic or biochemical hypoglycemia. Follow-up was continued until consistent satisfactory home monitoring and a fall in HbA_{1c} of >3% had been achieved, at which point patients were readmitted for another hypoglycemic clamp procedure performed according to the same profile as before.

Laboratory assays

Blood glucose was measured using a glucose oxidase method (Yellow Springs glucose analyzer, Yellow Springs Instruments, Yellow Springs, OH). Catecholamines were measured by high-performance liquid chromatography (24). Cortisol was measured by radioimmunoassay as described previously (25). Growth hormone and glucagon were measured using commercial radioimmunoassay kits (GH, NETRIA, London, U.K.; Glucagon, Diagnostic Products, Los Angeles, CA). Intra-assay variation for all assays used was <10%, and all samples from first and second clamps from one patient were assayed in the same batch.

Statistical analysis

Except for demographic data, which are expressed as means ± SD, all results are expressed as means ± SE. Plasma glucose thresholds for hormonal responses were determined in two ways: *1*) in statistical terms, as the blood glucose level at which a given hormone concentration on two or more consecutive samples exceeded by >2 SD the mean of five baseline measurements made during the initial euglycemic step, and 2) as a change in hormone level of known physiological significance. This change was defined as a rise over the baseline that occurred in at least two consecu-

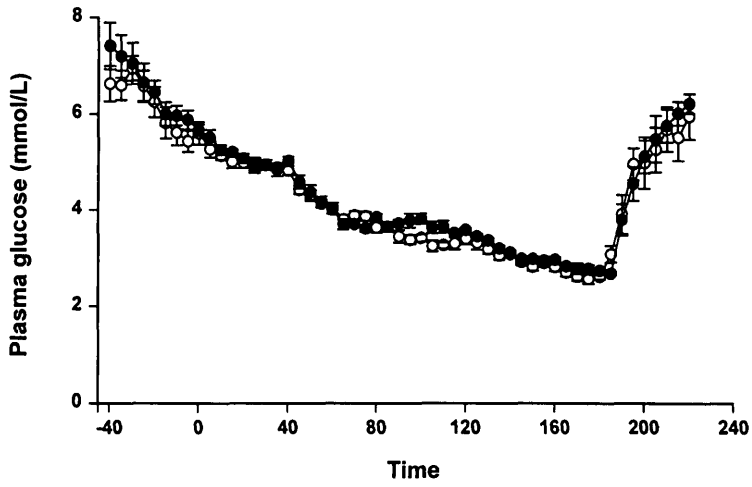

Figure 1—*Plasma glucose profiles during hyperinsulinemic clamps of type 2 patients. Time data are expressed in minutes. Points represent mean ± SE of seven subjects.* ●, *first clamp (poor glycemic control);* ○, *second clamp (improved glycemic control).*

tive samples and was >0.44 pmol/l for epinephrine, >0.33 pmol/l for norepinephrine, >192 nmol/l for cortisol, and >18 mU/l for growth hormone (26,27).

Magnitude of hormonal responses was assessed in terms of the peak hormone levels attained and as area under the curve of hormone levels plotted against time.

Thresholds for the development of autonomic and neuroglycopenic symptoms were arbitrarily defined as the plasma glucose levels at which symptom scores increased by ≥2 points over baseline on two consecutive assessments, based on the observation that these scores do not change during euglycemic hyperinsulinemic clamping in normal volunteers. Autonomic symptom scores were calculated by summing individual scores of sweating, anxiety, tremor, palpitations, and feeling hot. Neuroglycopenic scores were calculated from the scores for difficulty in speaking, confusion, dizziness, irritability, and drowsiness (28–30).

Thresholds for impairment of four-choice reaction time were defined as the plasma glucose levels at which there were two or more consecutive increments of ≥10% over the mean of three measurements made at euglycemia. Deterioration in the accuracy of reaction was also assessed and was deemed to have occurred when there was a sustained 4% or more increase in error rate. These definitions were based on previous repeated observations made over 4 h in healthy volunteers using the same apparatus, from which coefficients of variation of 5% in reaction time and 2% in accuracy were determined (14).

Results obtained from the first and second clamp studies of patients were compared using Student's paired *t* test. Comparisons between patients and nondiabetic subjects were made using Student's unpaired *t* test.

RESULTS

Metabolic control

All seven patients achieved improvement in glycemic control after transfer to insulin. Mean HbA_{1c} fell from 11.3 ± 1.1 to 8.1 ± 0.9% during a period of 127 ± 36 days between first and second clamp studies. No episodes of severe hypoglycemia occurred, and no patients recorded plasma glucose of <3.6 mmol/l on more than two occasions in the 2 weeks before the second clamp, despite their checking their glucose levels four times a day. This improvement in glycemic control was achieved at the expense of a mean weight gain of 6.4 ± 2.6 kg. One patient developed new background retinopathy during the course of the study, but there was no evidence of progression of retinopathy in the three patients who had background changes at recruitment.

Clamp glucose profiles

Mean plasma glucose profiles of patients obtained in first and second clamp studies are shown in Fig. 1. There were no significant differences between plasma glucose levels at any of the glucose plateau steps or at nadir between first and second studies of patients, or between patients and control subjects.

Baseline hormone measurements

No significant differences were observed in any basal hormone measurements between the first and second clamp studies of diabetic patients. The baseline cortisol levels in diabetic patients were significantly higher than those in nondiabetic men (360 ± 106 vs. 270 ± 56, $P = 0.032$), whereas basal norepinephrine and epinephrine levels did not differ significantly. Changes in the assay methods used prevented direct comparison

Table 2—*Plasma glucose thresholds for counterregulatory hormone responses to stepped hypoglycemia in type 2 diabetes patients before and after improving glycemic control compared with data for nondiabetic men*

	Statistically derived response			Clinical response		
	Type 2 diabetes patients			Type 2 diabetes patients		
	Poor glycemic control	Improved glycemic control	Nondiabetic men	Poor glycemic control	Improved glycemic control	Nondiabetic men‡
Norepinephrine	3.7 ± 0.1	3.3 ± 0.3	3.3 ± 0.2	3.7 ± 0.1	3.3 ± 0.1*	3.4 ± 0.2*
Epinephrine	4.4 ± 0.2	3.5 ± 0.3†	3.7 ± 0.2*	4.1 ± 0.2	3.4 ± 0.3†	3.4 ± 0.1†
Glucagon	3.5 ± 0.3	3.0 ± 0.2*	2.9 ± 0.1	—	—	—
Growth hormone	4.2 ± 0.2	3.2 ± 0.2†	3.4 ± 0.1*	3.6 ± 0.2	3.0 ± 0.1*	3.2 ± 0.2*
Cortisol	3.7 ± 0.2	2.8 ± 0.1†	3.6 ± 0.1	3.4 ± 0.1	2.7 ± 0.1†	2.9 ± 0.2*

Data are means ± SE of seven subjects and are expressed in millimoles per liter. Statistically derived values are >mean + 2 SD. *$P < 0.05$; †$P < 0.01$ vs. poorly controlled type 2 diabetes patients. ‡Data are from Matyka et al. (14).

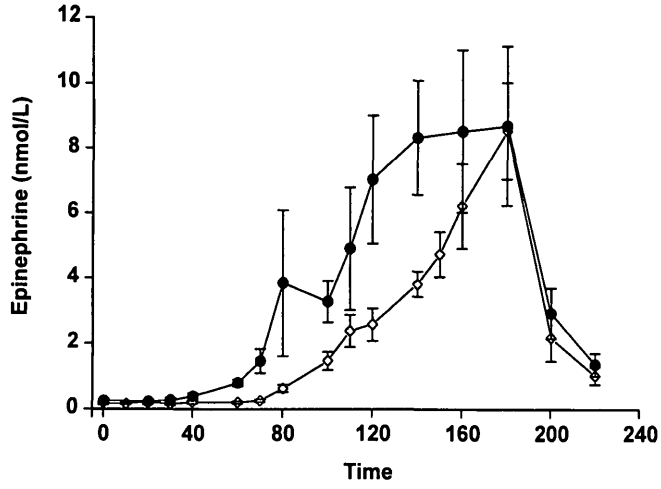

Figure 2—*Epinephrine responses to hypoglycemia in poorly controlled type 2 patients (●) and in nondiabetic men (◇). Time data are expressed in minutes.*

of glucagon and growth hormone levels of diabetic patients with those of nondiabetic subjects.

Counterregulatory hormone responses

In poorly controlled patients, counterregulatory hormone responses began at euglycemic levels, with significantly higher plasma glucose levels for the onset of clinically relevant elevation of epinephrine, norepinephrine, and growth hormone responses than in the nondiabetic comparison group (Table 2). For epinephrine and growth hormone, these differences were statistically significant when thresholds were determined according to both definitions described above, but the difference in norepinephrine thresholds was significant only when determined in terms of changes considered clinically relevant. For epinephrine, the area under hormone response curves (Fig. 2) was significantly greater in poorly controlled patients (690 ± 107 vs. 391 ± 57 nmol · min^{-1} · l^{-1}, $P = 0.027$).

After improvement in glycemic control, all counterregulatory hormone responses began later, at significantly lower plasma glucose levels than in the first study. The plasma glucose concentrations at the onset of the epinephrine, growth hormone, and cortisol responses were significantly lower after improved glycemic control, irrespective of the definition of the response, whereas the difference in norepinephrine thresholds was significant only when determined in terms of changes considered clinically relevant. Area under response curves for epinephrine and cortisol (Table 3, Fig. 3) were significantly reduced after improved glycemic control, although the reduction in peak response attained significance only for cortisol (Table 3). Growth hormone response began at a significantly lower plasma glucose than in the first study, although area under response curve and peak response did not differ significantly between the first and second clamps.

A modest response of glucagon to hypoglycemia was observed in five out of seven patients. In these five patients, there was a significant change in the threshold, but not in the magnitude, of this response after improved glycemic control (Tables 2 and 3). The remaining two patients had lower basal glucagon levels and absent glucagon response to hypoglycemia.

Symptom responses

In parallel with the change in hormonal responses were the profound effects of improved glycemic control on the onset and magnitude of the symptomatic responses to the hypoglycemic challenge. After glycemic control was improved, symptoms developed later and were less intense (Fig. 4). The plasma glucose thresholds at which total and autonomic scores increased were significantly different between the first and second clamps (Table 4). Area under response curves for both neuroglycopenic and autonomic symptoms were significantly lower after improved glycemic control (Table 5). It is noteworthy that in poorly controlled patients, the plasma glucose concentrations at the onset of symptom responses (total and autonomic) were significantly higher than in the nondiabetic comparison group and were restored to values of the nondiabetic group after improved glycemic control.

Four-choice reaction time

In initial hypoglycemic clamp studies, four-choice reaction time and accuracy of reaction deteriorated in all seven diabetic patients at a mean plasma glucose threshold of 3.1 mmol/l. These thresholds did not differ significantly from those observed previously in nondiabetic men (Table 4). After improvement in glycemic control, there were no significant changes in thresholds for deterioration in four-choice reaction time or accuracy of reaction (Table 4). During the second study, one patient did not demonstrate slowing of reaction time. This patient achieved a nadir of 2.8 mmol/l

Table 3—*Hormonal responses to stepped hypoglycemia in type 2 diabetes patients before and after improving glycemic control*

	Area under hormone response curve (per 180 min)		Peak response	
	Poor glycemic control	Improved control	Poor glycemic control	Improved control
Norepinephrine (nmol/l)	446 ± 57	445 ± 29	4.3 ± 0.6	4.0 ± 0.4
Epinephrine (nmol/l)	690 ± 107	306 ± 93*	11.7 ± 6.3	6.3 ± 1.2
Glucagon (nmol/l)	15,564 ± 2,314	14,425 ± 2,124	94.1 ± 16.3	98.6 ± 17.8
Growth hormone (mU/l)	4027± 735	3,015 ± 793 *	52.1 ± 8.1	50.8 ± 12.0
Cortisol (nmol/l)	89,586. ± 7,049	62,514 ± 6,583†	744 ± 34	572 ± 51*

Data are means ± SE of seven subjects. *$P < 0.05$; †$P < 0.01$.

(used in statistical calculations) with a deterioration in accuracy of reaction at 3.0 mmol/l in both first and second studies. Exclusion of this patient's data did not affect the statistical analysis. There was a significant change in the order in which responses to hypoglycemia occurred after improved glycemic control. Whereas symptoms preceded the onset of deterioration in four-choice reaction time in six out of seven patients in initial studies (mean difference between thresholds for symptom response and deterioration in four-choice reaction time 0.4 ± 0.1 mmol/l, P = 0.01), only one patient showed a symptom response before the onset of deterioration in four-choice reaction time after achieving improved glycemic control.

CONCLUSIONS — We have demonstrated that in patients with poorly controlled type 2 diabetes of long duration, counterregulatory hormone responses begin at a higher plasma glucose level and are brisker than in healthy subjects, with serum epinephrine reaching levels of clinical relevance while plasma glucose remains in the euglycemic range. This study represents the first demonstration of significant counterregulatory hormone responses at normoglycemia in type 2 diabetes.

Our data are consistent with those of Shamoon et al. (20), who demonstrated enhanced epinephrine responses in nine poorly controlled type 2 diabetes patients during mild hypoglycemia (nadir, 3.4 mmol/l). As in our study, these patients had higher mean basal cortisol levels than nondiabetic control subjects, but the magnitude of the cortisol response did not differ significantly between patients and control subjects.

Other studies of counterregulatory hormone responses in type 2 diabetes have shown conflicting results. In three studies of responses to hypoglycemia after intravenous insulin administration (16,18,19), no difference between patients and control subjects was observed in epinephrine responses to hypoglycemia. However, in each of these studies, glucose was lowered rapidly in a single step to nadir values ranging from 1.7 to 2.4 mmol/l. This experimental design would mask the exaggerated early epinephrine response we observed, because epinephrine levels were identical at nadir in poorly controlled patients and control subjects. Bolli et al. (17), using subcutaneous insulin administration to produce a slow fall in the blood glucose level to a nadir of 3.4 mmol/l, observed no difference in epinephrine responses between type 2 patients and control subjects, but cortisol and growth hormone responses were attenuated. In Bolli's study, patients were treated with diet alone, with a sulfonylurea drug, or with insulin, thus constituting a heterogeneous group, and the effects of previous glycemic experience were not considered.

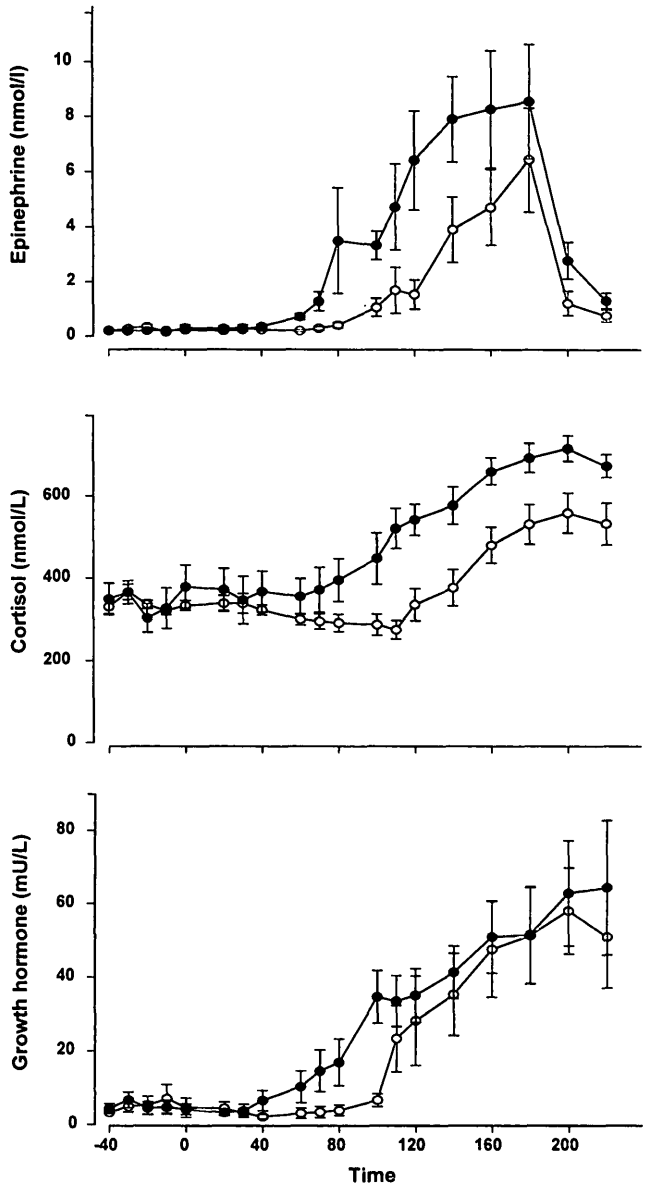

Figure 3—*Epinephrine, cortisol, and growth hormone responses to hypoglycemia in type 2 patients before and after improving glycemic control. Time data are expressed in minutes.* ●, *first clamp (poor glycemic control);* ○, *second clamp (improved glycemic control).*

A common defect in counterregulation observed in type 1 diabetes is the lack of glucagon responses (31–33). In type 2 diabetes, glucagon responses to hypoglycemia have been variably reported as being reduced or similar in magnitude to those observed in nondiabetic control subjects (16,17,20). The patients in our study had had their diabetes for several years and had reached the stage at which exogenous insulin is considered to be required for adequate metabolic control. Nevertheless, they all retained evidence of residual insulin secretion and the clinical and biochemical features of type 2 disease. In two of our patients, glucagon responses to hypoglycemia were absent; our data on the other five patients suggest that these responses are generally preserved in late type 2 diabetes.

Although our comparative nondiabetic data are from an older, all-male group, this

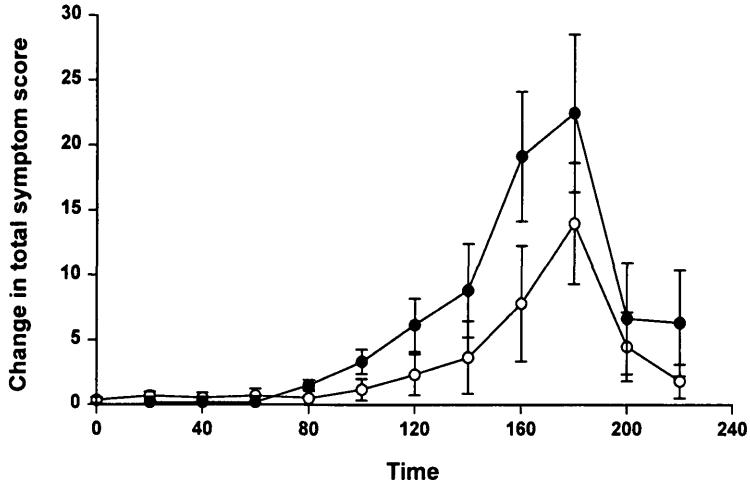

Figure 4—*Change in total symptom scores during hypoglycemia in type 2 patients before and after improving glycemic control. Time data are expressed in minutes.* ●, *first clamp (poor glycemic control);* ○, *second clamp (improved glycemic control).*

fact is unlikely to have affected our interpretation of the differences in counterregulatory hormone responses. In a previous study using an identical experimental protocol, we showed that the magnitude of epinephrine responses and the plasma glucose thresholds at which they begin do not change with normal aging (14). Other authors have described an age-related reduction in the magnitude of epinephrine response, but only in considerably older subjects than those of our control group (34) or as a subtle difference at an intermediate plasma glucose concentration of 3.3 mmol/l (35). In women, the magnitude of counterregulatory hormone responses is smaller than in men (36); thus, the inclusion of two women in the patient group would be expected to reduce, rather than contribute to, the differences observed.

We also observed an earlier onset of hypoglycemic symptoms in poorly controlled patients compared with nondiabetic volunteers, which was in keeping with the differences in counterregulatory hormone responses. In part, this observation may reflect the age difference between the patients and the nondiabetic volunteers, because the symptoms of hypoglycemia do diminish with age (14,15). However, the 10-year age gap between the two groups is considerably smaller than that for which an effect of age on symptom responses has been demonstrated. Furthermore, the glucose levels at which symptoms were reported by our diabetic patients in the present study were higher than those quoted in a study of healthy volunteers with a mean age of 30 years (15). Therefore, it seems reasonable to conclude that the symptom responses in our group of patients with poorly controlled diabetes are exaggerated beyond the expected response for healthy people of the same age.

After improved glycemic control, all counterregulatory hormone and symptom responses in our diabetic subjects began later, at glucose levels significantly lower than had been required in the same subjects during poor glycemic control and similar to those observed in nondiabetic men. Furthermore, the epinephrine, growth hormone, and cortisol responses were of significantly decreased magnitude. These results parallel, but do not mirror, the effects of intensified treatment in type 1 diabetes, after which counterregulatory hormone and symptom responses have been shown to be delayed and diminished (37–39). While recurrent hypoglycemia appears to be an important factor leading to the diminution of responses in type 1 diabetes, we observed changes in counterregulatory responses in the absence of detected hypoglycemia. However, whereas intensive treatment in type 1 diabetes is associated with a change from normal to subnormal levels, our data showed a normalization of initially exaggerated responses.

We used four-choice reaction time as a marker of cognitive function in our study. This test is very sensitive to acute hypoglycemia, showing deterioration in performance at plasma glucose levels of ~3 mmol/l (10,40–43), which compares favorably with other cognitive tests (43–47). It is thus a good marker of cognitive function (48) and, in particular, measures a function that is likely to be relevant to the performance of complex tasks such as driving (21,22). It is important to recognize that four-choice reaction time is not (nor is it intended as) an estimate of global cognitive function.

In contrast to its effects on hormonal responses and symptoms, glycemic control had no effect on glycemic thresholds for deterioration in four-choice reaction time or accuracy of reaction. With poor control, symptoms preceded the onset of cognitive impairment as the plasma glucose level fell, but with improved control, symptoms and deterioration in four-choice reaction time occurred simultaneously. Poorly controlled type 2 diabetes patients may thus be relatively protected from severe hypoglycemia

Table 4—***Plasma glucose thresholds for symptom responses and deterioration in four-choice reaction time during stepped hypoglycemia in type 2 diabetes patients before and after improving glycemic control, compared with data for nondiabetic men***

	Type 2 diabetes patients		
	Poor glycemic control	Improved glycemic control	Nondiabetic men†
Symptoms			
Autonomic	3.4 ± 0.1	2.8 ± 0.1*	2.8 ± 0.2*
Neuroglycopenic	3.2 ± 0.2	2.8 ± 0.2	2.8 ± 0.1
Combined	3.6 ± 0.1	3.0 ± 0.2*	3.0 ± 0.2*
Four-choice			
Time	3.1 ± 0.1	2.9 ± 0.1	3.0 ± 0.2
Accuracy	3.1 ± 0.1	3.0 ± 0.2	2.8 ± 0.1
First change	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.2

Data are means ± SE of seven subjects and are expressed in millimoles per liter. *$P < 0.05$ vs. poorly controlled type 2 diabetes patients. †Data are from Matyka et al. (14).

Table 5—*Area under symptom response curves in type 2 diabetes patients before and after improving glycemic control*

Symptoms	Poor glycemic control	Improved glycemic control
Autonomic	1,733 ± 181	1,400 ± 120*
Neuroglycopenic	1,630 ± 117	1,420 ± 106*
Combined	3,363 ± 287	2,820 ± 207*

Data are means ± SE of seven subjects. *$P < 0.05$.

by an enhanced symptom response for their age and by preservation of the normal hierarchy of responses to hypoglycemia seen in younger subjects (49), which is lost after modest improvement in glycemic control.

In summary, we have demonstrated exaggerated hormonal responses and early symptoms of hypoglycemia in poorly controlled type 2 diabetes, which normalize with improvement in glycemic control. Although early onset of symptoms has been demonstrated in poorly controlled type 1 diabetes (50), the development of counterregulatory hormone responses at euglycemia in adults has not been previously reported. The observed changes in responses after improved control are consistent with the hypothesis that the onset and magnitude of counterregulatory hormone and symptom responses depend on recent antecedent glycemic experience. An alternative explanation would be that the observed changes resulted from an effect of insulin treatment other than improved glycemic control, but this explanation seems unlikely because recent data have shown no difference in thresholds for counterregulatory responses between patients treated with insulin and those treated with a sulfonylurea drug (51). Finally, although there are no specific data on the reproducibility of responses to hypoglycemic challenge repeated after a period of months in type 2 diabetic patients, experience from repeated studies in type 1 diabetes would suggest that it is unlikely that the observed attenuation of responses observed in our studies could result either from the passage of time alone or from any residual effect of the first clamp (40,46).

We conclude that when patients with type 2 diabetes have poor glycemic control, they are relatively protected from severe hypoglycemia by exaggerated responses to their falling blood glucose levels. With modest improvement in glycemic control, these responses are diminished, closing the gap between the onset of symptoms and at least some cognitive impairment. Achieving optimal glycemic control in type 2 diabetic patients who require insulin is therefore likely to be associated with an increased risk of asymptomatic and potentially severe hypoglycemia.

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References

1. Heller SR, Cryer PE: Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes* 40: 223–226, 1991
2. Widom B, Simonson DC: Intermittent hypoglycemia impairs glucose counterregulation. *Diabetes* 41:1597–1602, 1992
3. Dagogo-Jack S, Craft S, Cryer PE: Hypoglycemia associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest* 91:819–828, 1993
4. Kerr D, Macdonald IA, Tattersall RB: Patients with type 1 diabetes adapt acutely to sustained mild hypoglycemia. *Diabet Med* 8:123–128, 1991
5. Davis M, Shamoon H: Counterregulatory adaptation to recurrent hypoglycemia in normal humans. *J Clin Endocrinol Metab* 73:995–1001, 1991
6. Mellman MJ, Davis MR, Brisman M, Shamoon H: Effect of antecedent hypoglycemia on cognitive function and on glycemic thresholds for counterregulatory hormone secretion in healthy humans. *Diabetes Care* 17:183–188, 1994
7. George E, Harris N, Bedford C, Macdonald IA, Hardisty CA, Heller SR: Prolonged but partial impairment of the hypoglycemic physiological responses following short-term hypoglycemia in normal subjects. *Diabetologia* 38:1183–1190, 1995
8. Amiel SA, Pottinger RC, Archibald HR, Chusney G, Cunnah DC, Prior PF, Gale EAM: Effect of antecedent glucose control on cerebral function during hypoglycemia. *Diabetes Care* 14:109–118, 1991
9. Hepburn DA, Patrick AW, Eadington DW, Ewing DJ, Frier BM: Unawareness of hypoglycemia in insulin treated diabetic patients: prevalence and relationship to autonomic neuropathy. *Diabet Med* 7:711–717, 1990
10. Maran A, Lomas J, Macdonald IA, Amiel SA: Lack of preservation of higher brain function during hypoglycemia in patients with intensively treated IDDM. *Diabetologia* 38:1412–1418, 1995
11. Mokan M, Mitrakou A, Veneman T, Ryan C, Korytkowski M, Cryer P, Gerich J: Hypoglycemia unawareness in IDDM. *Diabetes Care* 17:1397–1403, 1994
12. Gold AE, MacLeod KM, Frier BM: Frequency of severe hypoglycemia in patients with impaired awareness of hypoglycemia. *Diabetes Care* 17:697–703, 1994
13. Hepburn DA, MacLeod KM, Pell ACH, Scougal IJ, Frier BM: Frequency and symptoms of hypoglycemia experienced by patients with type 2 diabetes treated with insulin. *Diabet Med* 10:231–237, 1993
14. Matyka K, Evans M, Lomas J, Cranston I, Macdonald I, Amiel S: Altered hierarchy of protective responses against severe hypoglycemia in normal aging in healthy men. *Diabetes Care* 20:135–141, 1997
15. Brierly EJ, Broughton DL, James OFW, Alberti KGMM: Reduced awareness of hypoglycaemia in the elderly despite an intact counterregulatory response. *Q J Med* 88:439–445, 1995
16. Boden G, Soriano M, Hoeldtke RD, Owen OE: Counterregulatory hormone release and glucose recovery after hypoglycemia in non-insulin dependent diabetic patients. *Diabetes* 32:1055–1059, 1983
17. Bolli GB, Tsalikian E, Haymond MW, Cryer PE, Gerich JE: Defective counterregulation after subcutaneous insulin in non–insulin-dependent diabetes mellitus. *J Clin Invest* 73:1532–1541, 1984
18. Polonsky KS, Herold KC, Gilden JL, Bergenstal RM, Fang VS, Moossa AR, Jaspan JB: Glucose counterregulation in patients after pancreatectomy: comparison with other clinical forms of diabetes. *Diabetes* 33:1112–1119, 1984
19. Heller SR, Macdonald IA, Tattersall RB: Counterregulation in type 2 (non–insulin-dependent) diabetes mellitus: normal endocrine and glycemic responses up to ten years after diagnosis. *Diabetologia* 30:924-929, 1987
20. Shamoon H, Friedman S, Canton C, Zacharowicz L, Hu M, Rossetti L: Increased epinephrine and skeletal muscle responses to hypoglycemia in NIDDM. *J Clin Invest* 93:2562–2571, 1994
21. Wilkinson RT, Houghton D: Portable four-choice reaction time test with magnetic

tape memory. *Behav Res Meth Instrumentation* 7:441–446, 1975
22. Herbert M, Healy TEJ, Bourke JB, Fletcher IR, Rose JM: Profile of recovery after general anaesthesia. *Br Med J* 286:1539–1542, 1983
23. Liu D, Moberg E, Kollind K, Lins P-E, Macdonald IA: Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinemic euglycemia and hypoglycemia. *Diabetologia* 35:287–290, 1992
24. Macdonald IA, Lake DM: An improved method for extracting catecholamines from body fluids. *J Neurosci Meth* 13:239–248, 1985
25. Cunnah D, Jessop DS, Besser GM, Rees LH: Measurement of corticotrophin releasing factor in man. *J Endocrinol* 113:123–131, 1987
26. Clutter WE, Bier DM, Shah SD, Cryer PE: Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. *J Clin Invest* 66:94–101, 1980
27. Hiene RJ: Methods of investigation of insulin induced hypoglycemia. In *Hypoglycemia and Diabetes.* Frier BM, Fisher BM, Eds. London, Edward Arnold, 1993, p. 165–175
28. Hepburn DA, Deary ID, Frier BM, Patrick AW, Quinn JD, Fisher BM: Symptoms of acute hypoglycemia in humans with and without IDDM: a factor analysis approach. *Diabetes Care* 14:949–957, 1991
29. Hepburn DA, Deary ID, Frier BM: Classification of symptoms of hypoglycemia in insulin treated diabetic patients using factor analysis: relationship to hypoglycemia unawareness. *Diabet Med* 9:70–75, 1992
30. Deary ID, Hepburn DA, MacLeod KM, Frier BM: Partitioning of the symptoms of hypoglycemia using multiple sample confirmatory factor analysis. *Diabetologia* 36:772–777, 1993
31. Gerich JE, Langlois M, Noacoo C, Karam JH, Forsham PH: Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic α-cell defect. *Science* 182:171–173, 1973
32. Benson JW, Johnson DG, Palmer JP, Werner PL, Ensinck JW: Glucagon and catecholamine secretion during hypoglycemia in normal and diabetic men. *J Clin Endocrinol Metab* 44:459–464, 1977
33. Bolli G, De Feo P, Compagnucci P, Cartechini MG, Angeletti G, Santeusanio F, Brunetti P, Gerich JE: Abnormal glucose counterregulation in IDDM: interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. *Diabetes* 32:134–141, 1983
34. Meneilly GS, Cheung E, Tuokko H: Altered responses to hypoglycemia of healthy elderly people. *J Clin Endocrinol Metab* 78:1341–1348, 1994
35. Ortiz-Alonso FJ, Galecki A, Herman WH, Smith MJ, Javquez JA, Halter JB: Hypoglycemia counterregulation in elderly humans: relationship to glucose levels. *Am J Phys* 267:E497–E506, 1994
36. Amiel SA, Maran A, Powrie J, Umpleby AM: Gender differences in counterregulation to hypoglycaemia. *Diabetologia* 36: 460–464, 1993
37. Simonson DC, Tamborlane WV, DeFronzo RA, Sherwin RS: Intensive insulin therapy reduces the counterregulatory hormone responses to hypoglycaemia in patients with type 1 diabetes. *Ann Intern Med* 103:184–190, 1985
38. Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV: Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. *Diabetes* 37: 901–907, 1988
39. Amiel SA, Tamborlane WV, Simonson DC, Sherwin RS: Defective glucose counterregulation after strict control of insulin-dependent diabetes mellitus. *N Engl J Med* 316:1376–1383, 1987
40. Maran A, Lomas J, Archibald H, Macdonald IA, Gale EAM, Amiel SA: Double blind clinical and laboratory study of hypoglycemia with human and porcine insulin in diabetic patients reporting hypoglycemia unawareness after transferring to human insulin. *BMJ* 306:167–171, 1993
41. Maran A, Cranston I, Lomas J, Macdonald IA, Amiel SA: Protection by lactate of cerebral function during hypoglycemia. *Lancet* 343:16–20, 1994
42. Cranston I, Lomas J, Maran A, Macdonald IA, Amiel SA : Restoration of hypoglycemia awareness in patients with long-duration insulin-dependent diabetes. *Lancet* 344: 283–287, 1994.
43. Hvidberg AM, Fanelli CG, Hershey T, Terkamp C, Craft S, Cryer PE: Impact of recent antecedent hypoglycemia on hypoglycemic cognitive dysfunction in nondiabetic humans. *Diabetes* 45:1030–1036, 1996
44. Widom B, Simonson DC: Glycemic control and neurophysiological function during hypoglycemia in patients with insulin dependent diabetes mellitus. *Ann Intern Med* 112:904–912, 1990
45. Draelos MT, Jacobson AM, Weinger K, Widom B, Ryan CM, Finkelstein DM: Cognitive function in patients with IDDM during hyperglycemia and hypoglycemia. *Am J Med* 98:135–143, 1995
46. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Scionti L, Santeusanio F, Brunetti P, Bolli GB: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 42:1683–1689, 1993
47. Fanelli CP, Pampanelli S, Epifano L, Rambotti A, Ciofetta M, Modarelli F, Di Vincenzo A, Annibale B, Lepore M, Lalli C, Del Sindaco P, Brunetti P, Bolli G: Relative roles of insulin and hypoglycemia on induction of neuroendocrine responses to, symptoms of and deterioration of cognitive function in hypoglycemia in male and female humans. *Diabetologia* 37:797–807, 1994
48. Heller SR, Macdonald IA: The measurement of cognitive function during acute hypoglycemia: experimental limitations and their effect on the study of hypoglycemia unawareness. *Diabet Med* 13:607–615, 1996
49. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms and cerebral dysfunction. *Am J Physiol* 260:E67–E74, 1991
50. Boyle PJ, Schwartz NS, Shah SD, Clutter WE, Cryer PE: Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and in non-diabetics. *N Engl J Med* 318:1487–1492
51. Peacey SR, Bedford C, Marlow S, Harris N, Hardisty CA, Macdonald IA, Heller SR: Impaired peripheral physiological responses to hypoglycemia in insulin treated type 2 diabetes (Abstract). *Diabetologia* 39 (Suppl. 1):A66, 1996