A Reliable and Reproducible Test for Adequate Glucose Counterregulation in Type I Diabetes Mellitus

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SUMMARY

The safety, reproducibility, and reliability of an insulin infusion test for assessment of adequate glucose counterregulation were evaluated in 18 patients with type I (insulin-dependent) diabetes mellitus. When the test (a 60-min, 30-mU/m²/min insulin infusion) was administered on three separate occasions at 3–4-wk intervals, coefficients of variation for plasma glucose and counterregulatory hormone (glucagon, epinephrine, cortisol, and growth hormone) responses averaged less than 8%. No patient experienced symptoms requiring discontinuation of the test and plasma glucose concentrations increased spontaneously after stopping the insulin infusion. Using objective criteria based on plasma glucose nadirs or postnadir rates of plasma glucose recovery, no patient judged to have adequate glucose counterregulation by the test (postnadir rates of plasma glucose recovery or plasma glucose nadir above 0.4 mg/dl/min and 45 mg/dl) developed severe hypoglycemia (plasma glucose < 40 mg/dl) during up to 7 mo of intensive insulin therapy, whereas nearly all patients with inadequate counterregulation did. We conclude that this test, when performed in standardized conditions, is safe and reproducible and can reliably predict those patients with type I diabetes who are at risk of developing severe hypoglycemia during intensive insulin therapy.

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The hope that near-normalization of glycemic control may prevent or diminish the development of the long-term complications of diabetes mellitus has led to increasing use of intensive insulin therapy in patients with type I diabetes.[1] However, since most patients with type I diabetes have impaired glucose counterregulation,[2–4] there is considerable concern that indiscriminate use of intensive insulin therapy may provoke severe hypoglycemia in some patients.[5] It would, thus, be advantageous to have a means to identify, beforehand, patients who are at risk of developing severe hypoglycemia during intensive insulin therapy.

An intravenous insulin infusion test has recently been proposed for this purpose.[6] Using subjective (symptoms of neuroglycopenia irrespective of the plasma glucose concentration) or objective (plasma glucose below 40 mg/dl that did not subsequently increase) criteria for inadequate glucose counterregulation, White et al. showed that patients with inadequate glucose concentrations during the test subsequently had an increased frequency of severe hypoglycemic reactions during intensive insulin therapy.[6] These observations suggest that such a test may be useful in identifying patients with type I diabetes for whom standard intensive insulin therapy may not be appropriate. However, since the test of White et al.[6] can itself lead to severe neuroglycopenia, one would prefer a test in which less insulin is infused, while having a comparable predictive power of identifying patients at risk of severe hypoglycemia during intensive therapy. Moreover, the reproducibility of the test has not been established.

The present studies were therefore undertaken for the following purposes: (1) to determine whether a more convenient test that employed objective criteria could reproducibly characterize glucose counterregulation in patients with type I diabetes, (2) to determine whether the results of such a test correlated with patients' daily blood glucose concentrations at home, and (3) to determine whether the test could predict patients at risk of developing hypoglycemia during intensive insulin therapy.

MATERIALS AND METHODS

Subjects. Informed consent was obtained from 18 insulin-dependent patients without residual endogenous insulin secretion, as assessed by the glucagon stimulation test,[7] who were on conventional (nonoptimized) insulin therapy, and

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TABLE 1
Clinical features of subjects studied

Subjects	Age (yr)	Diabetes duration (yr)	Weight (% ideal body weight)	Usual insulin therapy		MGB* (mg/dl)	HbA_1 (%)
				Injections/day	U/kg/day		
Diabetic patients							
1	33	0.6	95	2 (AR + L; AR + L)†	0.41	180	12.3
2	16	0.3	105	1 (L)	0.35	203	14.7
3	21	0.4	102	2 (AR + L; AR + L)	0.43	175	11.1
4	18	0.8	113	2 (AR + L; AR + L)	0.29	181	12.4
5	26	1.4	91	1 (L)	0.35	195	13.7
6	17	0.7	99	1 (AR + L)	0.45	204	14.3
7	25	4	102	2 (L + L)	0.50	198	13.1
8	38	6	94	2 (L + L)	0.48	179	12.6
9	27	3	105	2 (AR + L; L)	0.37	180	13.9
10	42	6	106	1 (L)	0.59	175	13.7
11	31	5	97	2 (L + L)	0.41	166	12.0
12	33	4	89	2 (AR + L; L)	0.48	178	13.4
13	26	3	95	2 (L + L)	0.43	159	10.2
14	42	18	108	2 (AR + L; L)	0.51	155	10.9
15	36	12	113	2 (L + L)	0.39	162	11.4
16	29	14	97	1 (L)	0.47	192	13.4
17	39	21	104	2 (L + L)	0.38	184	12.2
18	45	19	101	2 (AR + L; AR + L)	0.31	161	10.5
Mean ± SEM	30 ± 2	6.6 ± 2	101 ± 2	—	0.42 ± 0.2	179 ± 3.5	12.5 ± 0.3
Normal subjects (N = 8)	29 ± 2	—	98 ± 1	—	—	104 ± 4	6.8 ± 0.1

*Mean blood glucose (two blood glucose profiles in the week before admission; data from home blood glucose monitoring).
†AR, Actrapid MC; and L, Lente insulins (Novo).

from 8 age- and weight-matched normal volunteers (Table 1). The diabetic subjects had no clinical evidence of autonomic neuropathy, as indicated by the normality of their beat-to-beat variation in heart rate during deep breathing[8] and lack of orthostatic hypotension (defined as fall in systolic blood pressure > 30 mm Hg).[9]

Protocols. Study 1. To assess the reproducibility of the test, all subjects underwent three insulin infusion tests (see below) separated by 20–30 days. During this period, the patients' therapeutic regimens were not altered. Fortuitously, four of the diabetic patients (nos. 8, 10, 11, and 13) had been studied previously (3–6 mo before the present studies) with the insulin infusion test 36–48 h after emergency admissions for acute episodes of diabetic ketoacidosis (DKA, plasma glucose concentration > 250 mg/dl, arterial pH < 7.10, urinary ketones + + +). This permitted the results of the insulin infusion test immediately after recovery from ketoacidosis to be compared with the results from the other three tests in these patients to evaluate the effects of glycemic control.

Study 2. To determine the correlation of the results of the test with blood glucose concentrations of patients at home and to prospectively evaluate the usefulness of the insulin infusion test as a predictor of severe hypoglycemia during intensive insulin therapy, all of the above diabetic subjects were placed on an intensive insulin regimen that consisted of three daily injections of insulin (regular insulin [Actrapid MC, Novo Industries, Copenhagen, Denmark] at breakfast, regular insulin at lunch, and regular plus lente insulin [Novo] at dinner) to optimize their glycemic control. Patients were treated for up to 7 mo (174 ± 4 days, range 119–196 days) and their frequency of hypoglycemic episodes was determined during home blood glucose monitoring. This was performed by means of a Dextrometer (Ames Division, Miles Laboratories, Elkhart, Indiana): daily fasting blood glucose concentrations and blood glucose profiles (blood glucose 30 min before and 90 min after meals and at midnight) were recorded twice or three times a week in each patient. In addition, the patients checked their blood glucose concentration any time they experienced symptoms suggestive of hypoglycemia. The percent of circulating ketoamine-HbA_1 (henceforth referred to as simply HbA_1) was determined at monthly intervals.

Insulin infusion test. Long-acting insulin was discontinued at least 48 h before the study and regular insulin was injected with each meal. On the evening before the test, an intravenous infusion of regular insulin (Actrapid MC U-40, diluted to ≈0.8 U/ml in 0.9% NaCl containing 0.5% human serum albumin, Immuno S.P.A., Pisa, Italy) was started and continued overnight by means of a syringe pump (Model 2681, Harvard Apparatus Co., Millis, Massachusetts). Adjustments, based on blood glucose measurements every 5–30 min by means of a Dextrometer, were made to maintain blood glucose concentration between 80 and 90 mg/dl until the beginning of the experiment the next morning. Nondiabetic subjects were admitted to the metabolic unit on the morning of the study.

All tests were begun between 9:30 a.m. and 10:30 a.m., with the diabetic and nondiabetic subjects having fasted overnight. The protocol chosen for the insulin infusion test consisted of an insulin infusion regimen similar to the one that we have used in several previous studies.[2,10–13] Regular insulin (Actrapid MC, diluted as above) was infused at the rate of 30 mU/m^2/min for 60 min; 50% of the basal insulin infusion rate was used from 60 throughout 150 min. Baseline

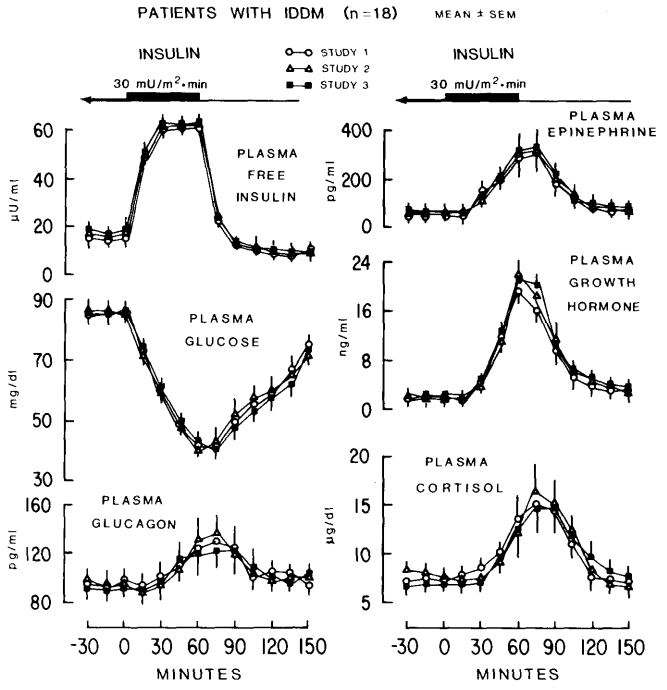

FIGURE 1. Reproducibility of plasma glucose and counterregulatory hormone responses during 3 insulin infusion tests carried out at 20–30-day intervals in 18 patients with type I diabetes.

venous blood samples were taken at −15 and 0 min and at 15-min intervals over the next 150 min.

Analytic methods. Venous blood samples were obtained for measurement of the concentrations of plasma glucose (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, California), insulin,[14] C-peptide[15] (after plasma extraction with polyethylene glycol), glucagon,[16] epinephrine,[17] norepinephrine,[17] growth hormone,[18] and cortisol[19] by previously described methods. Glycosylated hemoglobin concentration was determined after removal of the labile fraction.[20]

Statistical methods and calculations. All data are expressed as mean ± SEM. Paired and, when appropriate, unpaired Student's *t* tests were used for evaluation of statistical significance.[21] The rate of plasma glucose recovery was calculated from the time interval between plasma glucose nadir and return to baseline. In those diabetic subjects in whom the postnadir plasma glucose concentration did not reach baseline values within 150 min, the glucose increase to the 150-min value was employed. Patients having values for plasma glucose nadirs below the lowest value found in normal subjects, or rates of plasma glucose recovery that were 3 SD below the mean values for normal subjects, were considered to have abnormal glucose counterregulation.

RESULTS

Reproducibility of glucose counterregulation during the insulin infusion test (Figure 1, Tables 2 and 3). Blood glucose concentrations in the diabetic patients (mean of two blood glucose profiles during the week before each insulin infusion test: 179 ± 4 mg/dl, 191 ± 11 mg/dl, and 187 ± 10 mg/dl, P = NS) and plasma insulin concentrations achieved during each test were virtually identical. The diabetic patients, as a group, had impaired glucose counterregulation as indicated by plasma glucose nadirs and rates of postnadir plasma glucose recovery, which were significantly lower than corresponding values in the nondiabetic subjects (Table 2). However, diabetic subjects (nos. 1–6) with a short duration of diabetes (< 1.5 yr) had normal glucose counterregulation, whereas those with a longer duration of diabetes (nos. 7–18) showed impaired counterregulation (Table 2). Plasma glucose and counterregulatory hormone responses during each test were highly reproducible, as indicated by the high coefficients of correlation and the low coefficients of variation found between the results of each test (Table 3).

Effects of antecedent glycemic control (comparison of results of the insulin infusion test immediately after recovery from DKA) (Figure 2). During the insulin infusion test carried out soon after DKA, plasma glucose concentrations at the nadir (77 ± 7 mg/dl) and at 150 min (133 ± 12 mg/dl) were greater than respective values during the other three tests (37 ± 4 and 69 ± 7 mg/dl, P < 0.05). After DKA, baseline plasma glucagon (125 ± 8 versus 92 ± 3 pg/ml, P < 0.05), epinephrine (103 ± 13 versus 51 ± 7 pg/ml, P < 0.05), cortisol (9.4 ± 0.2 versus 6.4 ± 0.1 μg/dl, P < 0.05), and growth hormone (4.1 ± 1.2 versus 1.3 ± 0.5 ng/ml, P < 0.05) were all greater than values observed before

TABLE 2
Plasma glucose and counterregulatory hormone responses in normal and diabetic subjects*

	Normal subjects	Diabetic subjects		
	(N = 8)	(nos. 1–18)	(nos. 1–6)	(nos. 7–18)
Plasma glucose nadir (mg/dl)	48 ± 1	41 ± 2†	49 ± 2	37 ± 2†
Rate of plasma glucose recovery (mg/dl/min)	0.55 ± 0.02	0.37 ± 0.03†	0.55 ± 0.03	0.29 ± 0.01†
Plasma glucagon response (AUC, ng/ml/150 min)	5.9 ± 0.2	2.4 ± 0.6†	5.7 ± 0.4	0.73 ± 0.12†
Plasma epinephrine response (AUC, ng/ml/150 min)	25.8 ± 2.3	20.1 ± 1.9	26.2 ± 2.5	17 ± 2†
Plasma growth hormone response (AUC, ng/ml/150 min)	908 ± 111	1101 ± 66	966 ± 112	1170 ± 77
Plasma cortisol response (AUC, ng/dl/150 min)	339 ± 36	254 ± 26	335 ± 38	214 ± 28†

*Mean of 3 insulin infusion tests; AUC, area under curve above baseline.
†P < 0.05 versus normal subjects.

TABLE 3
Coefficients of correlation of changes in plasma glucose and counterregulatory hormone response during insulin infusion tests in diabetic subjects

	Correlation coefficients			Coefficients of variation
	Study 1 vs. 2	Study 1 vs. 3	Study 2 vs. 3	
Plasma glucose nadir (mg/dl)	0.89†	0.89†	0.72*	6.7 ± 0.5
Rate of plasma glucose recovery (mg/dl/min)	0.79*	0.89†	0.57*	4.8 ± 0.4
Plasma glucagon response (AUC)	0.82†	0.85†	0.88†	5.1 ± 0.9
Plasma epinephrine response (AUC)	0.87†	0.93†	0.80*	7.3 ± 0.6
Plasma growth hormone response (AUC)	0.90†	0.91†	0.97†	9 ± 1.3
Plasma cortisol response (AUC)	0.90†	0.97†	0.88†	12.1 ± 1.6

*$2P < 0.01$.
†$2P < 0.001$.

the other three tests. During the test, plasma glucagon responses were greater after DKA while plasma cortisol and growth hormone increased similarly in all four tests. Thus, glucose counterregulation appeared to be more improved after a period of severe deterioration of glycemic control than during mere suboptimal glycemic control.

Relationships between the results of the insulin infusion tests and blood glucose levels during intensive insulin therapy. Intensive insulin therapy resulted in improved glycemic control, as indicated by the decrease in mean daily blood glucose (186 ± 8 mg/dl versus 135 ± 2 mg/dl, $P < 0.05$) and percent HbA_1 (12.5 ± 0.3 versus 9.6 ± 0.1%, $P < 0.05$). Daily insulin doses required to obtain improved glycemic control were greater than those used during nonoptimized therapy (0.62 ± 0.02 versus 0.42 ± 0.02 U/kg, $P < 0.05$).

In the whole group of diabetic subjects, a total number of 380 episodes of blood glucose values < 60 mg/dl were recorded, with a frequency of 0.70 ± 0.12 episodes/100 person-days. The diabetic subjects who had abnormal glucose counterregulation in terms of lower plasma glucose nadirs or slower postnadir rates of plasma glucose recovery during the insulin infusion tests (nos. 7–18) had a frequency of hypoglycemia (1.54 episodes/100 person-days) that was at least 14 times the frequency (0.11 episodes/100 patient-days) in the patients with normal glucose counterregulation (nos. 1–6). When the frequency of blood glucose values between 50 and 60 mg/dl, between 40 and 50 mg/dl, and below 40 mg/dl were determined in each patient, there was a significant inverse correlation between the frequency of each subnormal blood glucose value and both the plasma glucose nadir ($r = -0.65$, $r = -0.72$, $r = -0.66$, respectively, all $P < 0.01$) and the rates of plasma glucose recovery from hypoglycemia ($r = -0.83$, $r = -0.84$, $r = -0.86$, respectively, all $P < 0.01$) during the insulin infusion tests, despite the fact that blood glucose measurements using a reflectance meter can sometimes give unreliable results in the hypoglycemic range.

Evaluation of objective criteria for predicting the risk of hypoglycemia. Postnadir plasma glucose recovery rates that were more than 3 SD below the normal mean values (0.4 mg/dl/min) and plasma glucose nadirs below the lowest value observed in the normal subjects (45 mg/dl) were assessed as objective criteria for their ability to identify patients at risk of developing severe hypoglycemia during intensive insulin therapy.

As shown in Figure 3, all diabetic subjects who had a postnadir rate of plasma glucose recovery or a plasma glucose nadir above 0.4 mg/dl/min and 45 mg/dl never had a recorded blood glucose level during intensive insulin therapy below 50 mg/dl, whereas patients with rates of plasma glucose recovery or plasma glucose nadirs below those values frequently had blood glucose levels below 40 mg/dl. Thus, these objective criteria appear to be reliable for identifying patients who are at risk for developing hypoglycemia during intensive insulin therapy.

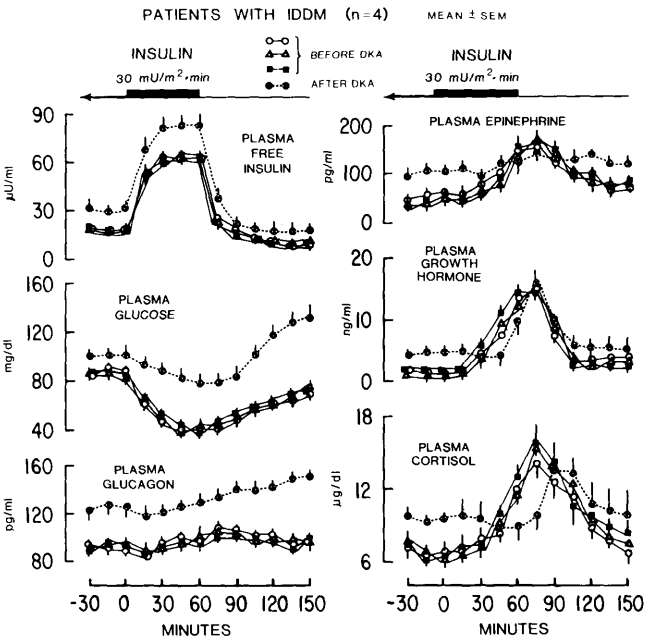

FIGURE 2. Plasma glucose and counterregulatory hormone responses to 3 insulin infusion tests carried out during suboptimal glycemic control (before DKA), and 36–48 h after an emergency admission for diabetic ketoacidosis in 4 patients with type I diabetes.

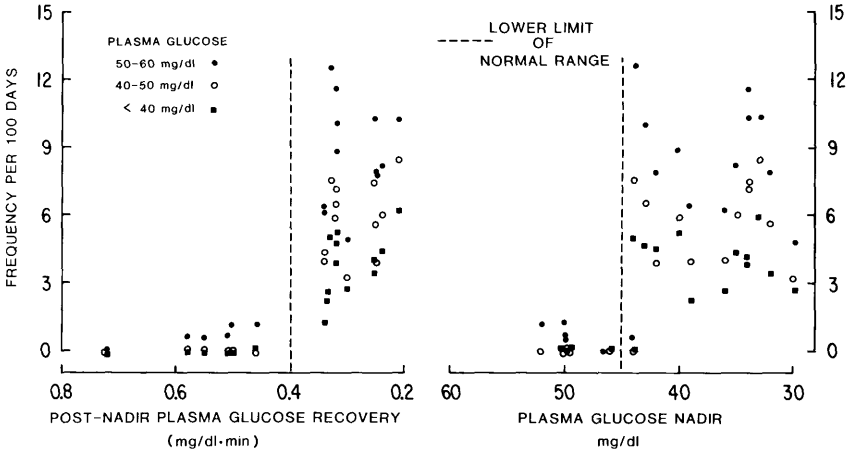

FIGURE 3. Relationship between frequency of hypoglycemia (home blood glucose monitoring) and plasma glucose nadir (right) and postnadir plasma glucose recovery (left) during the insulin infusion test in 18 patients with type I diabetes.

DISCUSSION

The present study demonstrates that an insulin infusion test, when performed under carefully controlled conditions, is a highly reproducible assessment of glucose counterregulation in patients with type I diabetes mellitus and can reliably predict patients at risk of developing severe hypoglycemia during intensive insulin therapy. When patients with type I diabetes were given the test on three separate occasions, plasma glucose and counterregulatory hormone responses were quite similar with coefficients of variation averaging less than 8%. Moreover, using objective criteria based on values for rates of postnadir plasma glucose recovery and plasma glucose nadir, the test was able to prospectively identify those diabetic patients who would not develop severe hypoglycemia (< 40 mg/dl) during intensive insulin therapy, and also those patients who were prone to have an increased frequency of abnormally low blood glucose levels (< 60 mg/dl).

Thus, our results support the conclusion of White et al.[6] that such a test should be of value in assessing which patients with type I diabetes are suitable candidates for intensive insulin therapy. Moreover, our study establishes objective criteria for discriminating between patients who are and are not prone to develop severe hypoglycemia.

It is important to point out certain differences between the insulin test used in the present study and that used by White et al.[6] White et al. infused insulin at a rate of 40 mU/kg/h for 90 min, which resulted in plasma free insulin levels in diabetic subjects > 100 μU/ml, whereas we infused insulin for only 60 min at a rate of 30 mU/m^2/min, which resulted in plasma insulin levels of approximately 60 μU/ml, as previously reported.[22] In the study of White et al., the test had to be discontinued in three patients because of symptoms of neuroglycopenia, and other patients had prolonged hypoglycemia. With our insulin infusion, the test did not have to be aborted in any patient; none of the patients had symptomatic neuroglycopenia and all patients had spontaneous recovery from hypoglycemia. Thus, use of our insulin infusion rate appears to be safer and better tolerated while being no less effective in identifying patients at risk of developing severe hypoglycemia during intensive insulin therapy.

We therefore recommend a 60-min insulin infusion at the rate of 30 mU/m^2/min with determinations of plasma glucose values at 15-min intervals up to 30, 45, 60, 75, and 90 min. Although the rate of postnadir plasma glucose recovery and plasma glucose nadir appeared to be equally effective parameters for predicting risk for hypoglycemia, we recommend use of the plasma glucose nadir, since, after it has been reached (usually before 75 min), the test can be terminated, thus shortening both the time required and any accompanying discomfort to patients.

It should be emphasized that the validity of this test requires strict attention to its performance under carefully controlled conditions, i.e., having baseline plasma glucose concentrations in the normoglycemic range (80–100 mg/dl), having patients withdraw from their intermediate- or long-acting insulin, and not performing the test on patients in severely poor control. Initial hyperglycemia or hypoglycemia would render the absolute criteria of the test meaningless. Moreover, persistence of the effect of previously injected intermediate- or long-acting insulin may falsely lead to the conclusion that glucose counterregulation was inadequate. Finally, since patients recently in very poor control may be resistant to insulin due to increased circulating levels of counterregulatory hormones, performance of the test on such patients may give the false impression of adequate glucose counterregulation. However, mere suboptimal glycemic control (HbA_1 up to 14%) or optimization of therapy should not affect the results of the test.[10]

In summary, when performed under standardized conditions, a 60-min, 30-mU/m^2/min infusion of insulin provides a safe and reproducible assessment of glucose counterregulation in patients with type I diabetes mellitus. This test appears to be able to identify, beforehand, patients at risk of developing severe hypoglycemia during intensive insulin therapy.

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