Rate of Glucose Fall Does Not Affect Counterregulatory Hormone Responses to Hypoglycemia in Normal and Diabetic Humans

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SUMMARY

To test the hypothesis that variations in rate of glucose fall influence counterregulatory hormone responses to hypoglycemia, we have modified the glucose-clamp technique to provide a reproducible hypoglycemic stimulus in normal and type I diabetic subjects that varied only in the rate of glucose fall. Responsive elevations in plasma epinephrine and norepinephrine and in growth hormone, glucagon, and cortisol were not significantly affected by a ninefold change in the rate at which plasma glucose was lowered from 83 ± 1 to 50 ± 1 mg/dl in normal subjects. Similarly, wide variation in the rate of fall produced no substantive differences in counterregulatory hormone responses to hypoglycemia in diabetic subjects. The plasma glucose threshold at which epinephrine release began, determined from the slow-fall studies, was 63 ± 3 mg/dl in normal subjects but exhibited a wide range (48–74 mg/dl). Similar values were found in the diabetics. Thresholds for growth hormone, cortisol, and glucagon were slightly lower, ranging from 45 to 68 mg/dl in the normals. Our data suggest that counterregulatory hormone responses to hypoglycemia are triggered by the glucose level per se and not by its rate of fall. Furthermore, individual differences in glucose thresholds for epinephrine release may contribute to variations in the glucose level associated with hypoglycemic symptoms. *Diabetes* 36:518–22, 1987

The precise nature of the variables determining the magnitude of the counterregulatory hormone responses to hypoglycemia is poorly defined. In particular, it has not been established whether the rate of glucose fall modulates the release of counterregulatory hormones when plasma glucose declines below the normal range. The clinical impression that slow rates of glucose fall may not produce adrenergic symptoms even in the presence of biochemical hypoglycemia (1,2) and vice versa (3,4) has led to the suggestion that hormonal responses may be influenced by the rate of glucose fall. However, this view is not universally accepted (5–8). Underlying the controversy is the lack of a study that specifically examines the effect of varying rates of glucose fall during the development of hypoglycemia. This may be due in part to practical difficulties in producing controlled and reproducible hypoglycemia in vivo.

In our study this obstacle was overcome by modification of the glucose-clamp technique to provide a uniform hypoglycemic stimulus in which rate of glucose fall was the only variable. By providing a standardized, gradual decline, these studies also allowed us to identify more precisely the glucose level (or threshold) at which counterregulatory hormone responses began.

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MATERIALS AND METHODS

Subjects. Two groups of subjects were studied. The first group consisted of 12 nonobese insulin-dependent diabetic patients (7 men and 5 women) who did not have a C-peptide response (≤0.2 pmol/ml) to 1 mg i.v. glucagon and/or a 24-h urinary C-peptide excretion of <8 ng/mg creatinine. Their mean age was 30 ± 2 yr (range 20–45), and duration of diabetes was 14 ± 2 yr (range 3–28). All were receiving conventional insulin therapy (1–2 daily injections of short- and/or intermediate-acting insulin, total dose 0.6 ± 0.1 $U \cdot kg^{-1} \cdot day^{-1}$) and were taking no other medications. The patient's usual insulin dose was omitted on the morning of study. All subjects had a normal plasma creatinine and creatinine clearance, and none had clinical evidence of diabetic neuropathy on physical examination. Their mean glycosylated hemoglobin was 11.2 ± 0.8% (normal range 5.0–8.0%). The second group consisted of 12 nonobese healthy volunteers (9 men and 3 women), aged 25 ± 2 yr (range 18–34). None had a history of reactive hypoglycemia or a

family history of diabetes or were taking any medications. Each subject habitually consumed a weight-maintaining diet containing at least 200 g carbohydrate/day.

The nature, purpose, and possible risk of the study were explained to each subject before written consent was obtained. The protocol was approved by the Yale Human Investigations Committee.

Procedures. All subjects were studied in the General Clinical Research Center at the Yale New Haven Medical Center after a 10- to 12-h overnight fast. Two intravenous catheters were inserted, one for infusion of glucose and insulin and the second, which was placed retrogradely in a vein draining the hand, for blood sampling. The hand bearing the sampling line was placed in a warming box (65°C) to arterialize venous blood (9,10). After the collection of at least three baseline blood samples, a primed continuous infusion of regular insulin (Lilly, Indianapolis, IN) was administered for 180 min at a maintenance rate of 1 mU · kg^{-1} body wt · min^{-1}. In nondiabetic controls, 20% dextrose (Travenol, Deerfield, IL) was infused from the outset at a variable rate based on frequent (every 5 min) plasma glucose determinations to regulate the glucose levels as described below. In diabetic subjects, glucose concentrations were allowed to decrease to 90 mg/dl before the dextrose infusion was begun. Thereafter, the same procedures were followed as in controls.

Slow-fall study. Plasma glucose was maintained at 80–90 mg/dl for the initial 40 min of the glucose clamp. The dextrose infusion rate was then reduced to produce a fall in plasma glucose of ~10 mg/dl every 40 min until plasma glucose reached 50 mg/dl. This final level of glycemia was maintained for 60 min. Additional blood samples were drawn every 10–20 min for measurement of counterregulatory hormones. Six normal and six diabetic subjects underwent the slow-fall protocol described above. Four additional normal subjects (3 men and 1 woman, aged 21–27 yr) completed a slow-fall study in which plasma glucose did not reach 50 mg/dl; these studies were terminated at glucose levels ranging from 55 to 58 mg/dl. Their data were used in the analysis of glucose thresholds because plasma glucose fell sufficiently to trigger counterregulatory hormone release. However, these studies were not used for quantitative comparison with the fast-fall study because the magnitude of the hypoglycemic stimulus was different.

Fast-fall studies. Euglycemia (80–90 mg/dl) was maintained for the initial 120 min of the glucose clamp. The dextrose infusion was then transiently discontinued, allowing plasma glucose to fall rapidly. Thereafter, the variable dextrose infusion was resumed to maintain plasma glucose at 50 mg/dl for 1 h. Eight normal and six diabetic subjects participated in this protocol.

Determinations. Total glycosylated hemoglobin was measured with a microcolumn kit (Isolab, Akron, OH). Plasma glucose was measured in duplicate on a Beckman II glucose analyzer (Fullerton, CA), the accuracy of which was checked at ~30-min intervals during each study with a standard glucose solution. The coefficient of variation of glucose determinations is <2%. Plasma epinephrine and norepinephrine were determined by a radioisotope enzymatic assay (Upjohn, Kalamazoo, MI) and plasma cortisol by a fluorimetric method (11). Plasma glucagon (12), growth hormone (13), and insulin (14) were measured by radioimmunoassay. For estimation of free insulin, plasma samples were immediately treated with polyethyleneglycol to precipitate antibody-bound insulin (15) and were assayed within 4 wk of the study.

Analyses and statistical methods. Comparisons of basal measurements were made with Student's *t* test for nonpaired observations. Hormonal responses to hypoglycemia were compared with two-way analysis of variance, and, where quoted, peak values represent the means of values between 150 and 180 min. The glucose threshold for counterregulatory hormone release was defined as the plasma glucose level at which circulating hormones rose by >2SD above basal. Basal values for this purpose include all samples obtained before plasma glucose was allowed to fall. A hormone rise was only considered significant if sustained for at least 20 min. All data are expressed as means ± SE.

RESULTS

Plasma glucose. The glucose profiles for the slow- and fast-fall studies in normal and diabetic subjects are shown in Fig.

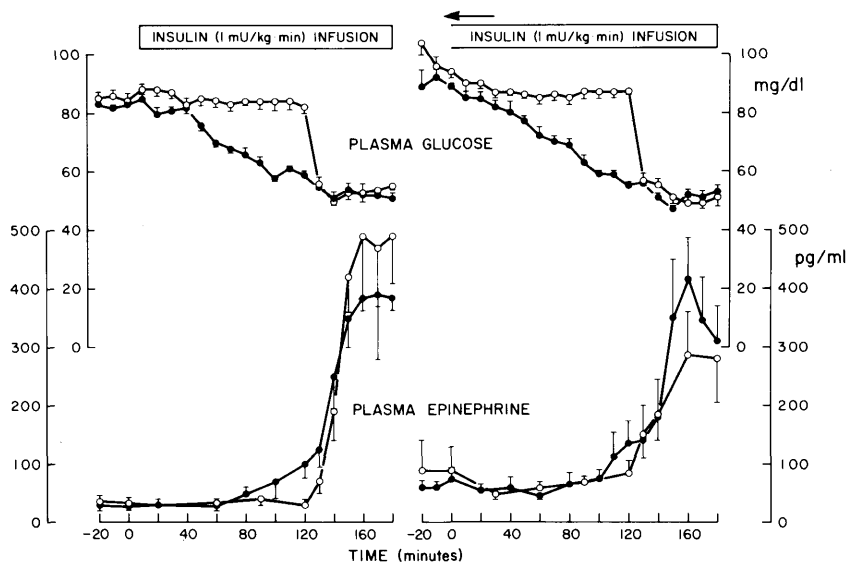

FIG. 1. Plasma glucose profiles (*top panels*) and epinephrine responses (*bottom panels*) to either slow-fall (●) or fast-fall (○) studies. Nondiabetics are shown on *left* and diabetics on *right*. Six normal and 6 diabetic subjects completed slow-fall studies; 8 normal and 6 diabetic subjects completed fast-fall studies.

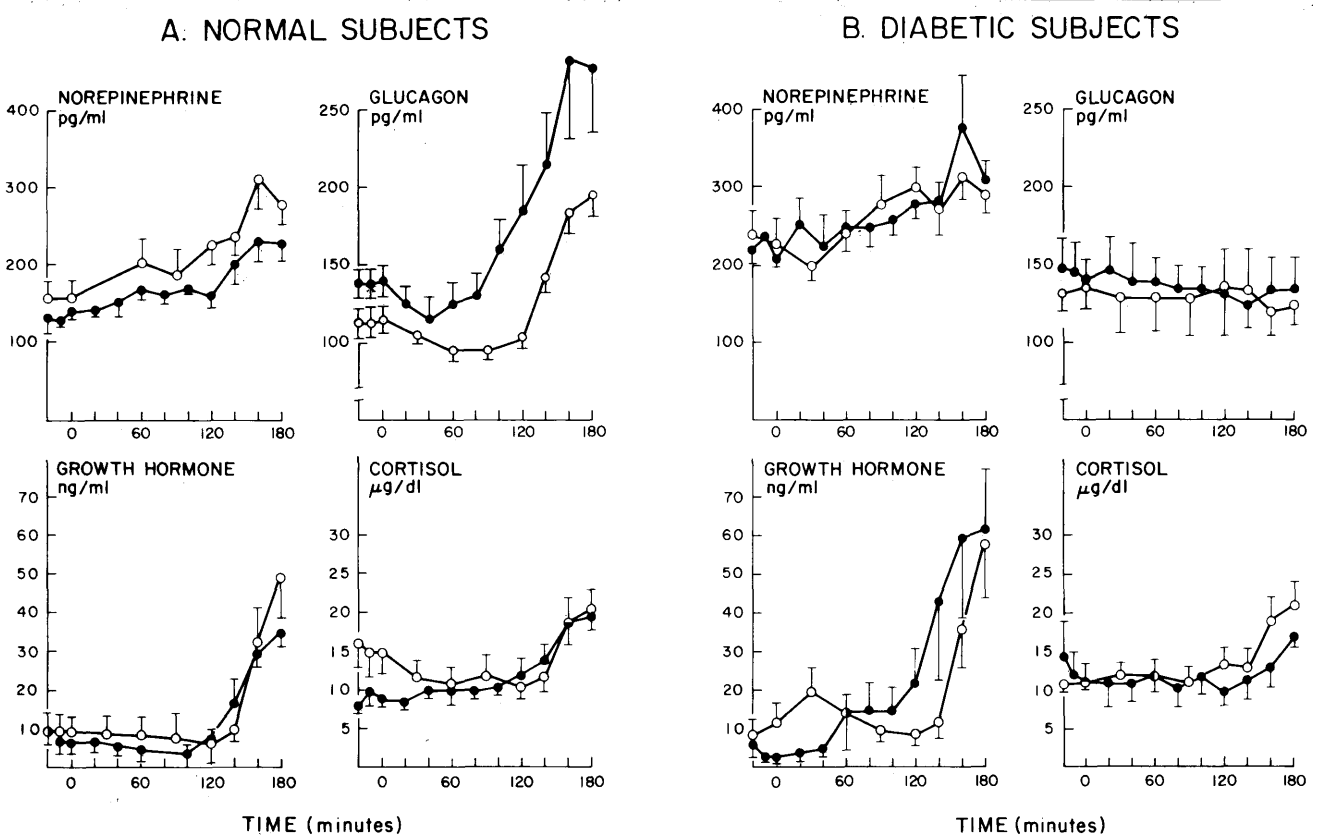

FIG. 2. *A*: counterregulatory hormone responses to slow (●, *n* = 6) and fast (○, *n* = 8) reduction in plasma glucose in normal subjects. *B*: counterregulatory hormone responses to slow (●, *n* = 6) and fast (○, *n* = 6) reduction in plasma glucose in diabetic subjects. From *top left*, clockwise in both *A* and *B*, profiles of norepinephrine, glucagon, cortisol, and growth hormone are shown.

1. In normal subjects, the glucose fall (83 ± 1 to 50 ± 2 mg/dl slow fall vs. 85 ± 1 to 50 ± 2 mg/dl fast fall) and the time point at which the glucose nadir occurred were the same in the slow- and fast-fall studies. The rate of glucose decline in the slow-fall study (0.31 mg · dl^{-1} · min^{-1}) was one-ninth that achieved in the fast-fall study (2.7 mg · dl^{-1} · min^{-1}). In the diabetics, the glucose profiles in the slow- and fast-fall studies followed a similar pattern. On the morning of the study, there was no significant difference between fasting plasma glucose of the slow-fall (133 ± 29 mg/dl) and fast-fall (127 ± 19 mg/dl, *P* not significant) studies. Thereafter, plasma glucose was reduced to ~90 mg/dl and then held between 80–90 mg/dl during the initial 40 min (slow fall) or 120 min (fast fall) of the study. Plasma glucose during the period of euglycemia (84 ± 2 vs. 87 ± 1) and at glucose nadir (50 ± 1 vs. 50 ± 2 mg/dl) was virtually identical in the two studies. Again, there was a ninefold difference in rate of glucose fall. During the insulin infusion, steady-state insulin levels in normal subjects (108 ± 3 μU/ml) were higher than the free-insulin levels (72 ± 13 μU/ml, $P < .025$) achieved in the diabetics.

Catecholamines. As shown in Fig. 1, epinephrine responses were not significantly different during the slow- and fast-fall studies. In normals (*bottom left panel*), plasma epinephrine rose from 28 ± 6 to 360 ± 58 pg/ml during the slow-fall (a significant rise being first detected at 120 min) and from 34 ± 6 to 470 ± 87 pg/ml during the fast-fall studies (*P* not significant). Similarly, in the diabetics (*bottom right panel*), peak epinephrine levels were similar in the two studies (341 ± 71 pg/ml slow fall vs. 301 ± 70 pg/ml fast fall, *P* not significant). There were no significant differences between the epinephrine responses of nondiabetic and diabetic subjects.

Norepinephrine responses are shown in Fig. 2 (*top left panels*) for normal subjects (*A*) and diabetic subjects (*B*). Although in the fast-fall study in normal controls baseline norepinephrine levels were higher and rose slightly during the euglycemic phase, the increment in plasma norepinephrine during hypoglycemia in this study (67 ± 18 pg/ml) was not different from that during the slow-fall study (86 ± 21 pg/ml, *P* not significant). In diabetic subjects (Fig. 2*B*), the norepinephrine responses were small and only achieved significance during the slow glucose fall. However, the final norepinephrine levels in the two studies in diabetic subjects were not significantly different.

Glucagon, growth hormone, and cortisol. Plasma profiles for glucagon, cortisol, and growth hormone during the slow- and fast-fall studies in nondiabetic and IDDM subjects are also shown in Fig. 2. In normal subjects, the increments in growth hormone and cortisol (Fig. 2*A*, *bottom panels*) were comparable in the slow- and fast-fall studies, whereas the rise in plasma glucagon (Fig. 2*A*, *top right panel*) was slightly greater during the slow-fall study (mean peak value 282 ± 42 vs. 192 ± 15 pg/ml, $P < .05$). As expected, glucagon did not rise significantly in either study in subjects with diabetes (Fig. 2*B*, *top right panel*). The cortisol response (Fig. 2*B*,

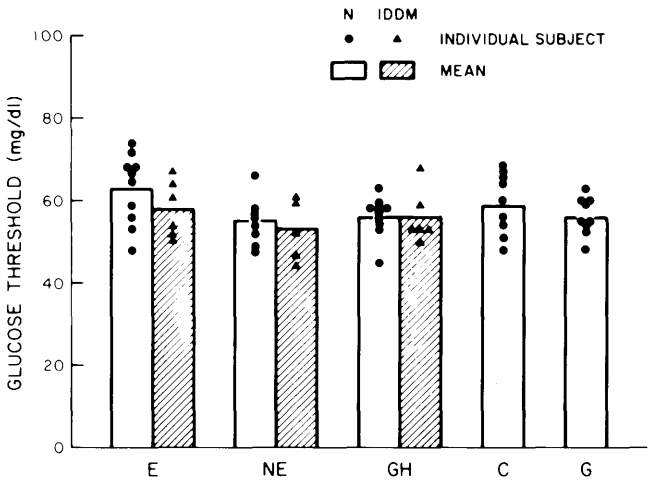

FIG. 3. Glucose thresholds for counterregulatory hormone release. *Bars* (*open*, nondiabetic; *hatched*, diabetic subjects) show mean plasma glucose at which each hormone had risen by >2SD above its basal value. ●, Individual nondiabetic subjects, *n* = 10; ▲, individual diabetic subjects, *n* = 6. E, epinephrine; NE, norepinephrine; GH, growth hormone; C, cortisol; G, glucagon.

bottom right panel) was also small in the diabetic subjects, but neither the cortisol peaks nor the change in cortisol levels was significantly different between the slow- and fast-fall studies. The growth hormone response in the diabetic subjects (Fig. 2*B*, *bottom left panel*) tended to be greater during the slower rate of glucose fall (peak values 58 ± 16 vs. 39 ± 10 ng/ml, slow vs. fast, *P* not significant), but again this difference was not statistically significant. Neither cortisol nor growth hormone responses differed significantly between diabetic and normal subjects.

Glucose threshold. The glucose levels at which counterregulatory hormone release began during the slow-fall study are shown in Fig. 3. The plasma glucose that triggered significant epinephrine release averaged 63 ± 3 mg/dl in controls and 58 ± 4 mg/dl in diabetics (*P* not significant). The release of the other hormones in nondiabetics was triggered by slightly lower plasma glucose levels, with mean thresholds ranging from 55 to 59 mg/dl (Fig. 3). For each hormone, the scatter of glucose thresholds among individual subjects (nondiabetic and diabetic) was wide, however. For example, significant epinephrine responses occurred at glucose levels of between 48 and 74 mM. The glucose threshold that triggers a response of epinephrine >200 pg/ml also varied, ranging from 47 to 65 mg/dl.

DISCUSSION

The rate of glucose fall is thought to influence the magnitude of the counterregulatory response to hypoglycemia and has been used to explain apparent discrepancies between subjective awareness of hypoglycemia and actual blood glucose level (1–4). Although this assumption has been disputed (6–8,16), the question has never been put to formal test. Previous studies have shown that a very rapid rate of glucose fall does not of itself stimulate a prominent hormonal response when plasma glucose remains above the normoglycemic range (6,7,16). However, in those studies, hormone responses were not compared under conditions where the rate of glucose fall was varied or where glucose nadir was the same. Furthermore, none of those studies evaluated whether a gradual reduction in blood glucose levels attenuates counterregulatory responses during hypoglycemia.

We varied rates of glucose fall by a factor of 9 during a reduction of plasma glucose to a single hypoglycemic level. We chose 50 mg/dl as a hypoglycemic stimulus because most investigators have used a more intense hypoglycemic stimulus (≤40 mg/dl) to provoke counterregulatory responses. Such profound hypoglycemia provides a maximal stimulus to counterregulatory mechanisms and thus could potentially obliterate any differences in hormone release due to more subtle factors, e.g., the rate of glucose fall. Previous studies have shown that a rapid reduction in plasma glucose to 50 mg/dl is sufficient to trigger demonstrable counterregulatory hormone responses in normal and diabetic subjects (17). We reasoned that this submaximal stimulus would be best able to reveal possible differences in the hormonal responses related to the rate of glucose fall. Furthermore, plasma glucose levels of 50 mg/dl are not uncommonly seen in patients with reactive hypoglycemia, insulinoma, or diabetes.

Our results show that catecholamine and other counterregulatory hormone responses to mild hypoglycemia are not diminished by a slow rate of glucose fall in either normal or diabetic subjects. Because the observed variations in the counterregulatory responses between the slow- and fast-fall studies were minor and inconsistent, it is unlikely that we failed to detect an effect of any clinical relevance. Yet it remains possible that a much slower rate of glucose fall, such as might occur overnight, may affect hormonal responses to hypoglycemia.

Note that with the exception of glucagon we could not demonstrate any statistically significant differences between normal and diabetic subjects in the magnitude of the hormone responses to hypoglycemia. The absence of a glucagon response in diabetes is well recognized (18,19). Some workers have also reported reduced epinephrine release during hypoglycemia in long-standing type I diabetes (20). However, this finding has not been consistently observed (7,21) and may reflect differences in neuropathic status or preceding glycemic control of the diabetic populations studied (17).

The high circulating plasma insulin levels achieved in this study were necessary to effectively regulate the rate of glucose fall. Whereas such hyperinsulinemia only occurs clinically during insulin overdosage or occasionally in patients with insulinoma and may have affected the hormonal responses quantitatively, note that the peak counterregulatory hormone responses seen here are not dissimilar from those reported by Sacca et al. (22) in a hypoglycemic study with a much smaller insulin dose (0.4 mU · kg^{-1} · min^{-1}). In any event, because the same dose of insulin was used in all subjects, the comparison of responses between the slow- and fast-fall studies should be valid.

The slow-fall study, by providing a gradual reduction in plasma glucose, allowed us accurately to identify the glucose concentration at which counterregulatory hormone release began. The mean glucose threshold for statistically significant epinephrine release in normal subjects was 63 ± 3 mg/dl. The other counterregulatory hormones were

released at slightly lower glucose values, in keeping with other observations of a hierarchy of hormone release that place catecholamines in a first-line position (7,21). Although these glucose thresholds for hormonal release may appear high, our results are consistent with data inferred from the literature. For example, Sacca et al. (22) found a rise in counterregulatory hormones during an insulin infusion that reduced plasma glucose to 55 ± 3 mg/dl but not during a lower-dose infusion that reduced venous plasma glucose to ~67 mg/dl. Furthermore, Glick (23) reported that the glucose threshold for growth hormone release was 52 mg/dl. Note that these studies used venous blood sampling, which would have underestimated the true glucose thresholds for counterregulation (9).

A striking finding was the range of glucose levels that triggered catecholamine responses; the plasma glucose level at which epinephrine release began ranged between 48 and 74 mg/dl. Substantial differences were also observed in the plasma glucose level (range 47–65 mg/dl) required to raise epinephrine concentrations >200 pg/ml, a level likely to produce symptoms in at least some patients (24). The wide range of glucose thresholds may contribute to the variability of symptoms among normal individuals during borderline hypoglycemia. A similarly wide range of glucose thresholds for epinephrine release was observed in diabetic subjects who were poorly controlled. Recent studies have suggested that improved glycemic control of diabetes may lower the glucose threshold, triggering counterregulatory hormone release (17,25). Thus, differences in preceding glycemic control among diabetics may enhance the variability of glucose thresholds for counterregulation to a greater extent than is reported here.

In summary, a fall in plasma glucose from 80–90 to 50 mg/dl produced a rise in counterregulatory hormones that was not significantly altered by wide differences in the rate of glucose fall and was triggered by a mean glucose level ranging from 47 to 65 mg/dl. These data suggest that variations in the magnitude of counterregulatory hormone responses to hypoglycemia may not be explained by variations in rate of glucose fall. Instead, they appear to be determined by the absolute plasma glucose level achieved, which shows considerable variation even among normal individuals. Such variation in hormonal responses may underlie the variation in occurrence of symptoms in people presenting with hypoglycemia.

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