Recurrent Hypoglycemia Does Not Impair the Cortisol Response to Adrenocorticotropin Infusion in Healthy Humans

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Previous studies have shown that hypoglycemia may reduce counterregulatory responses to subsequent hypoglycemia in healthy subjects and in patients with diabetes. The effect of hypoglycemia on the hormonal response to a nonhypoglycemic stimulus is uncertain. To test the hypothesis that the cortisol response to corticotropin (ACTH) infusion is independent of antecedent hypoglycemia, 10 healthy subjects received a standard ACTH infusion (0.25 mg Cosyntropin [Organon, West Orange, NJ] intravenously over 240 minutes) at 8:00 AM on day 1 and day 3 and a hypoglycemic insulin clamp study (1 mU/kg/min) at 8:00 AM on day 2. During the hypoglycemic clamp, plasma glucose decreased from 5.0 mmol/L to 2.8 mmol/L for two periods of 120 minutes (mean glucose, 2.9 ± 0.03 and 2.8 ± 0.02 mmol/L, respectively) separated by a 60-minute interval of euglycemia (mean glucose, 4.7 ± 0.01 mmol/L). Seven subjects also had paired control studies in random order during which a 330-minute euglycemic clamp (mean glucose, 5.0 ± 0.11 mmol/L) instead of a hypoglycemic clamp was performed on day 2. Basal ACTH (4.6 ± 0.7 *v* 2.6 ± 0.4 pmol/L, $P < .02$) and basal cortisol (435 ± 46 *v* 317 ± 40 nmol/L, $P < .02$) both decreased from day 1 to day 3 following intervening hypoglycemia. In contrast, with intervening euglycemia, neither basal ACTH (5.9 ± 1.5 *v* 4.5 ± 1.0 pmol/L) nor basal cortisol (340 ± 38 *v* 318 ± 60 nmol/L) were reduced significantly on day 3 compared with day 1. Following interval hypoglycemia, the area under the curve (AUC) for the cortisol response to successive ACTH infusions was increased (4,734 ± 428 nmol/L over 240 minutes [day 3] *v* 3,526 ± 434 nmol/L over 240 minutes [day 1], $P < .01$). The maximum incremental cortisol response was also significantly increased (805 ± 63 nmol/L (day 3) *v* 583 ± 58 nmol/L (day 1), $P < .05$). In contrast, the AUC for the cortisol response to successive ACTH infusions with interval euglycemia (3,402 ± 345 nmol/L over 240 minutes [day 3] *v* 3,709 ± 391 nmol/L over 240 minutes [day 1] and the incremental cortisol response (702 ± 62 nmol/L [day 3] *v* 592 ± 85 nmol/L [day 1] were unchanged. Following exposure to intermittent hypoglycemia in healthy humans, fasting morning ACTH and cortisol levels are reduced and the incremental cortisol response to an infusion of ACTH is enhanced. The enhanced cortisol response to exogenous ACTH infusion after intervening hypoglycemia (but not intervening euglycemia) may reflect priming of the adrenal gland by endogenous ACTH produced during the hypoglycemia. These data suggest that adrenal function testing by exogenous ACTH administration is not impaired by prior exposure to hypoglycemia. Moreover, the reduced cortisol response to recurrent hypoglycemia in patients with well-controlled diabetes is not likely the result of impaired adrenal responsiveness.

IN HEALTHY SUBJECTS, recovery from acute insulin-induced hypoglycemia is dependent on the production of glucagon and epinephrine.[1] Cortisol and growth hormone (GH) are involved in the recovery from prolonged hypoglycemia.[2,3] In subjects with insulin-dependent diabetes mellitus (IDDM), the glucagon response to hypoglycemia is impaired; therefore, in these subjects epinephrine is the primary counterregulatory hormone defense against acute hypoglycemia.[4] Further, strict glycemic control of IDDM is associated with reduced epinephrine, adrenocorticotropin (ACTH), cortisol, and symptomatic responses to hypoglycemia, and these responses occur at a lower glucose level.[5-11] The cause of the reduction in the counterregulatory response and glucose threshold appears to be exposure to recurrent episodes of hypoglycemia. Studies have shown that exposure to recurrent hypoglycemia may induce a blunting of glucagon, epinephrine, cortisol, and GH responses, as well as decreasing the threshold for release of these hormones, in both healthy subjects and subjects with IDDM.[12-16] A critical duration and severity of hypoglycemia may be necessary to induce these alterations in counterregulatory hormone responses.[17-20]

The mechanism for the reduction of the counterregulatory response is not yet known. However, central nervous system adaptation to recurrent hypoglycemia has been shown to occur in both healthy subjects and subjects with IDDM.[21-23] End-organ adaptation and hormonal depletion have also been suggested as potential peripheral mechanisms whereby recurrent hypoglycemia might induce a reduction in counterregulatory responses.[24]

It is also uncertain whether exposure to recurrent hypoglycemia affects hypothalamic-pituitary-adrenal responses to a non-hypoglycemic stimulus.[25-28] To test the hypothesis that the reduction in the cortisol response to hypoglycemia is stimulus-specific, we studied the cortisol response to ACTH infusion before and after a hypoglycemic stimulus. This model also allowed us to evaluate the effect of recurrent hypoglycemia on end-organ adaptation, using exogenous ACTH as a direct test of adrenal gland function.

SUBJECTS AND METHODS

Ten healthy subjects participated in the study (Table 1). They had no medical illnesses, were not taking medications, and did not have a

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Table 1. Demographic Characteristics of the Study Subjects

Characteristic	Value
No. of subjects	10
Age (yr)	26 ± 6
Sex (male/female)	4/6
BMI (kg/m²)	23 ± 2
Hemoglobin A_1 (%)	5.9 ± 0.5

NOTE. Values are the mean ± SD.
Abbreviation: BMI, body mass index.

family history of diabetes mellitus. Voluntary written informed consent was obtained from each subject before enrollment. The protocol was approved by the Joslin Diabetes Center Committee on Human Studies.

Subjects fasted for 10 to 12 hours before each study, and reported to the Clinical Research Center at Joslin Diabetes Center at 8:00 AM. On each of the 3 study days, a catheter was inserted into the antecubital vein for administration of test substances, and a second catheter was placed in a retrograde fashion into a vein on the dorsum of the ipsilateral hand or wrist for blood sampling. The hand was placed in a heated box (70°C) to ensure arterialization of venous blood.[29]

Seven of 10 subjects were studied for 3 consecutive days on two occasions. Each underwent both the hypoglycemic (HYPO) and euglycemic (EUG) studies in random order 4 weeks apart. Three subjects underwent only the HYPO study. The overall design was the same for both the HYPO and EUG series of studies.

Day 1

On day 1, 0.25 mg Cosyntropin (Organon, West Orange, NJ) in 250 mL normal saline was infused over 4 hours. Blood samples for ACTH, cortisol, glucose, catecholamines, insulin, glucagon, and GH were drawn at 30- to 60-minute intervals.

Day 2

Subjects underwent a hyperinsulinemic-hypoglycemic clamp (HYPO) or a hyperinsulinemic-euglycemic clamp (EUG).[30] For both studies, a primed continuous infusion (6 pmol/kg/min) of crystalline human insulin (Eli Lilly, Indianapolis, IN) was administered with a variable infusion of 20% dextrose to achieve the desired plasma glucose levels. During the HYPO study, plasma glucose was decreased from 5.0 mmol/L to 2.8 mmol/L for two periods of 120 minutes (HYPO 1 and HYPO 2) separated by a 60-minute interval of euglycemia (5.0 mmol/L). During the EUG study, glucose was maintained at 5.0 mmol/L for 330 minutes (Fig 1). Blood samples were taken at 5-minute intervals for glucose measurements and at 15- to 60-minute intervals for ACTH, cortisol, catecholamine, glucagon, GH, and insulin measurements.

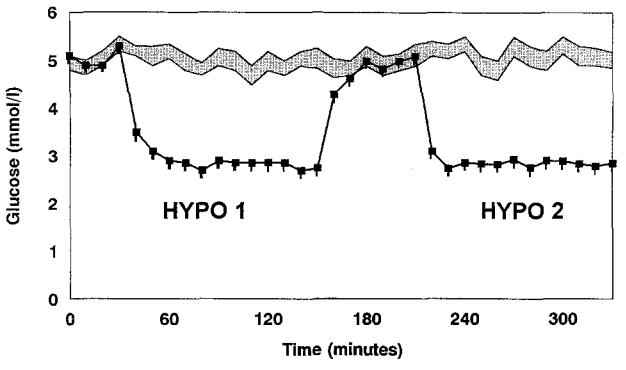

Fig 1. Plasma glucose levels during a hyperinsulinemic-hypoglycemic clamp study in 10 healthy subjects (■). Shaded area shows plasma glucose levels (mean ± SEM) in 7 of the same 10 subjects during a euglycemic control clamp.

Day 3

Following both HYPO and EUG studies, subjects underwent a 0.25-mg Cosyntropin infusion identical to that described for day 1.

The plasma glucose level was measured at the bedside by the glucose oxidase method with a glucose analyzer (YSI, Yellow Springs, OH). Plasma insulin levels were measured by a double-antibody radioimmunoassay (RIA).[31] Plasma epinephrine and norepinephrine levels were determined by radioenzymatic assay.[32] GH,[33] glucagon,[34] cortisol,[35] and ACTH[36] levels were determined using standard RIA procedures. Hemoglobin A_1 was assayed as described previously.[37]

Results are expressed as the mean ± SE unless otherwise indicated. Comparisons between groups were assessed by Student's *t* test for paired data or ANOVA as appropriate. All statistical analyses were performed using the SYSTAT statistical software program (Evanston, IL).

RESULTS

Hormonal Responses During HYPO and EUG Clamp Studies

Glucose and insulin. Fasting plasma glucose (5.1 ± 0.1 *v* 5.0 ± 0.1 mmol/L) and insulin (70 ± 5 *v* 79 ± 16 pmol/L) did not differ prior to the HYPO and EUG studies. The mean plasma glucose levels achieved during the two 120-minute periods of hypoglycemia (HYPO 1 and HYPO 2) were 2.9 ± 0.03 and 2.8 ± 0.02 mmol/L. The mean plasma glucose during EUG was 4.7 ± 0.01 mmol/L (Fig 1). Steady-state insulin levels did not differ during HYPO and EUG studies (646 ± 70 *v* 596 ± 28 pmol/L).

Glucagon. Glucagon levels tended to be lower during HYPO 2 compared with HYPO 1, but the differences did not reach statistical significance ($P = .056$ at time 0; Table 2).

Epinephrine and norepinephrine. Epinephrine levels during the HYPO study were significantly higher at all time points in HYPO 2 compared with HYPO 1 ($P < .05$ to .01; Fig 2A). However, peak incremental epinephrine levels did not differ significantly between HYPO 2 and HYPO 1 (1,401 ± 286 *v* 1,174 ± 191 pmol/L). Total and incremental norepinephrine levels during HYPO did not differ between HYPO 2 and HYPO 1 (Table 2).

Table 2. GH, Glucagon, and Norepinephrine Responses During Successive 120-Minute Episodes of Hypoglycemia (2.9 mmol/L) Separated by 60 Minutes of Euglycemia (5.0 mmol/L)

	Time (min)				
Parameter	0	30	60	90	120
GH (ng/mL)					
HYPO 1	2.0 ± 0.7*	1.9 ± 0.3*	6.0 ± 3*	16 ± 4	22 ± 3
HYPO 2	8.0 ± 3	7.0 ± 2.6	20 ± 5	25 ± 3	26 ± 4
Glucagon (pg/mL)					
HYPO 1	81 ± 14	107 ± 17	144 ± 19	148 ± 23	185 ± 29
HYPO 2	50 ± 5	119 ± 21	123 ± 28	122 ± 26	136 ± 29
Norepinephrine (nmol/L)					
HYPO 1	1.1 ± 0.1	1.4 ± 0.2	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.2
HYPO 2	1.5 ± 0.2	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.2

*$P < .05$, HYPO 1 *v* HYPO 2.

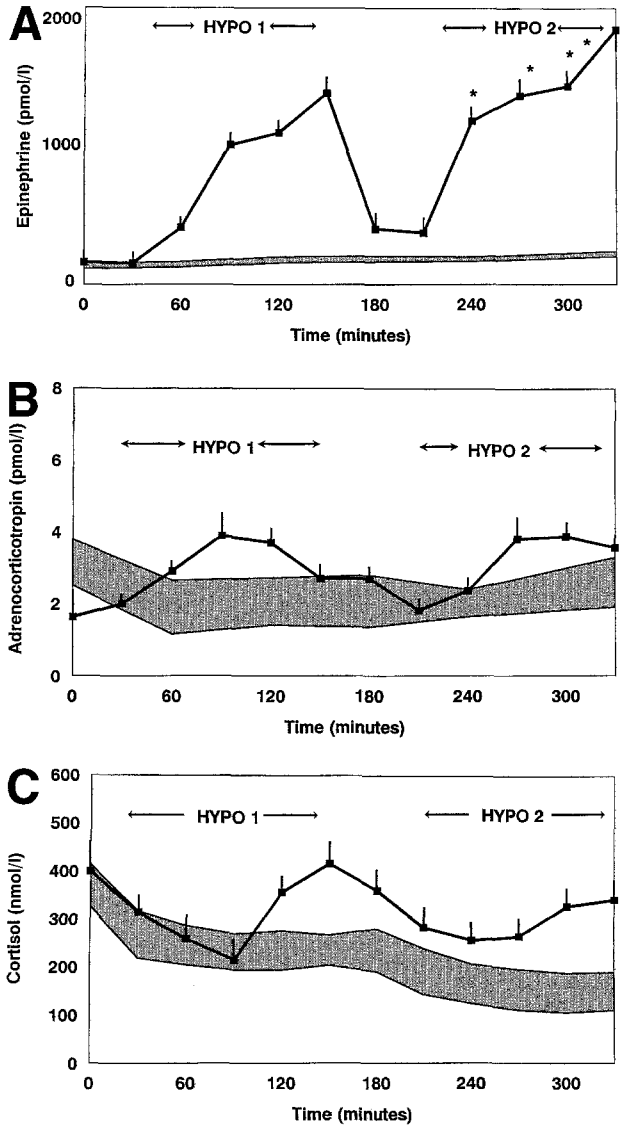

Fig 2. (A) Epinephrine levels during 2 episodes of hypoglycemia in 10 healthy subjects (■). Shaded area shows epinephrine levels (mean ± SEM) in 7 of the same 10 subjects during a euglycemic control clamp. *$P < .05$ to .01, HYPO 1 *v* HYPO 2. (B) ACTH levels during 2 episodes of hypoglycemia in 10 healthy subjects (■). Shaded area shows ACTH levels (mean ± SEM) in 7 of the same 10 subjects during a euglycemic control clamp. (C) Cortisol levels during 2 episodes of hypoglycemia in 10 healthy subjects (■). Shaded area shows cortisol levels (mean ± SEM) in 7 of the same 10 subjects during a euglycemic control clamp.

ACTH and cortisol. Basal ACTH levels were 3.1 ± 0.8 pmol/L before HYPO 1 and 2.9 ± 0.9 pmol/L before HYPO 2 (P = NS; Fig 2B). Peak ACTH levels (4.1 ± 1.3 *v* 3.9 ± 1.0 pmol/L) and mean ACTH levels (3.2 ± 0.2 *v* 4.9 ± 0.7 pmol/L) did not differ between HYPO 2 and HYPO 1 (Fig 2B). Basal cortisol levels were 355 ± 52 nmol/L before HYPO 1 and 309 ± 51 nmol/L before HYPO 2 (P = NS; Fig 2C). Peak cortisol levels were lower during HYPO 2 (341 ± 38 nmol/L) compared with HYPO 1 (416 ± 59 nmol/L), but this difference did not reach statistical significance.

GH. GH levels were significantly higher in HYPO 2 compared with HYPO 1 at the baseline, 30-minute, and 60-minute time points ($P < .05$; Table 2). However, peak GH levels (26 ± 4 *v* 22 ± 3 μg/L) and peak incremental GH levels (15 ± 6 *v* 19 ± 3 μg/L) did not differ significantly between HYPO 2 and HYPO 1.

Hormonal Responses During Infusions of ACTH

Glucose and insulin. Fasting plasma glucose levels did not differ significantly on day 3 versus day 1 with intervening HYPO (5.1 ± 0.1 *v* 5.1 ± 0.2 mmol/L) or EUG (4.9 ± 0.1 *v* 4.9 ± 0.1 mmol/L). Glucose levels were not significantly different at any time point during ACTH infusion (data not shown). Fasting insulin levels did not differ significantly on day 3 versus day 1 with intervening HYPO (68 ± 6 *v* 56 ± 5 pmol/L) or EUG (90 ± 23 *v* 58 ± 5 pmol/L).

Glucagon. Basal glucagon levels were lower (66 ± 8 *v* 100 ± 14 ng/L, $P = .05$) on day 3 compared with day 1 with intervening HYPO. There was no difference in basal glucagon (59 ± 18 *v* 58 ± 14 ng/L) on day 3 versus day 1 with intervening EUG.

ACTH and cortisol. Following interval HYPO, the basal level of ACTH was lower on day 3 (2.6 ± 0.4 pmol/L) than on day 1 (4.6 ± 0.7 pmol/L, $P < .02$). With intervening EUG, basal ACTH levels did not differ on day 3 (4.5 ± 1.0 pmol/L) versus day 1 (5.9 ± 1.5 pmol/L). Basal cortisol was also lower on day 3 (317 ± 40 nmol/L) than on day 1 (435 ± 46 nmol/L) with interval HYPO ($P < .02$). Basal levels of cortisol were unchanged on day 3 (318 ± 60 nmol/L) compared with day 1 (340 ± 38 nmol/L) following interval EUG.

With infusion of ACTH, the area under the curve (AUC) for cortisol was greater on day 3 (4,734 ± 428 mmol/L over 240 minutes) than on day 1 (3,526 ± 434 mmol/L over 240 minutes) with interval HYPO ($P < .01$). The AUC for cortisol did not differ on day 3 (3,709 ± 391 nmol/L over 240 minutes) compared with day 1 (3,402 ± 345 nmol/L over 240 minutes) with intervening EUG (Fig 3). In addition, on day 3 after intervening HYPO, the cortisol level at 240 minutes (1,122 ± 78 *v* 1,018 ± 71 nmol/L, $P < .05$) and the maximum increment in cortisol (805 ± 63 *v* 583 ± 58 nmol/L, $P = .02$) were signifi-

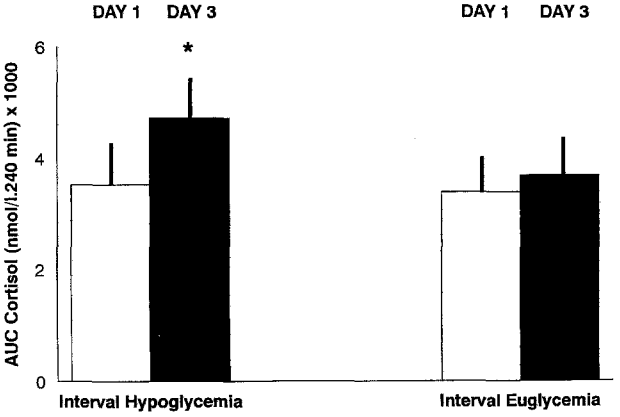

Fig 3. Cortisol AUC during a 240-minute ACTH infusion on day 1 (□) and day 3 (■) with intervening hypoglycemia (n = 10) or euglycemia (n = 7) on day 2. *$P < .05$, day 3 *v* day 1.

cantly greater on day 3 compared with day 1, respectively. However, on day 3 with intervening EUG, cortisol at 240 minutes (1,001 ± 90 *v* 931 ± 69 nmol/L) and the maximum incremental cortisol value (702 ± 62 *v* 592 ± 85 nmol/L) did not differ from the values on day 1.

Catecholamines and GH. Epinephrine, norepinephrine, and GH levels did not differ significantly on days 1 and 3 during ACTH infusion either with intervening HYPO or EUG (data not shown).

DISCUSSION

In this study, an ACTH infusion was used to evaluate the effect of prior exposure to recurrent hypoglycemia on the cortisol response to a nonhypoglycemic stimulus. Cortisol release is predominantly mediated by ACTH, and therefore, the ACTH infusion provided a direct measure of adrenal adaptation to recurrent hypoglycemia. We found that exposure to two episodes of hypoglycemia in healthy humans resulted in lower fasting morning levels of ACTH and cortisol and an enhanced incremental cortisol response to ACTH infusion.

Recurrent hypoglycemia may be associated with a reduction in counterregulatory hormone responses to subsequent hypoglycemia in both normal subjects and subjects with IDDM.[12-17] Previous studies would suggest there is a critical degree of exposure to hypoglycemia necessary to induce these adaptations in counterregulatory responses, and that the adaptation may be dependent on the duration, frequency, and severity of the hypoglycemic stress.[18-20] Widom and Simonson[14] demonstrated reductions in the epinephrine and cortisol responses following recurrent hypoglycemia to 2.2 mmol/L on 4 successive days in 10 healthy subjects. Heller and Cryer[12] demonstrated that a single 2-hour afternoon episode of hypoglycemia to 2.8 to 3.0 mmol/L was adequate to induce blunting of the epinephrine, cortisol, pancreatic polypeptide, and glucagon responses the next morning. Similarly, a single episode of asymptomatic hypoglycemia during sleep (160 minutes at 2.2 to 2.5 mmol/L) reduced epinephrine, cortisol, GH, and glucagon responses to hypoglycemia the next morning in healthy subjects.[15] Davis and Shamoon[13] reported significantly lower glucagon and GH responses and a trend toward a lower cortisol response to hypoglycemia in the second of two sequential 120-minute episodes of hypoglycemia to 3.0 mmol/L. Using the same model as Davis and Shamoon,[13] our results also show a trend toward lower glucagon and cortisol responses in the second of two sequential hypoglycemic episodes compared with the first; however, the epinephrine and GH responses remained intact. Taken together, it appears that a 2-hour episode of hypoglycemia to 3.0 mmol/L is sufficient to impair the cortisol response to subsequent hypoglycemia, and this effect persists through the next morning in healthy humans. We used this assumption in the current model, although the cortisol response to hypoglycemia was not directly tested the next morning in our subjects.

Few data exist regarding the effect of recurrent hypoglycemia on ACTH responses in healthy subjects. The ACTH response was not reduced during the second episode of hypoglycemia in our study. Lingenfelser et al[17] reported a nonsignificant reduction in the ACTH response to recurrent hypoglycemia in subjects with IDDM. However, these subjects were rendered hypoglycemic on 3 successive days between paired hypoglycemic insulin clamp protocols. Therefore, a critical degree of exposure to recurrent hypoglycemia may be necessary to induce the blunted counterregulatory responses, and this critical exposure may vary for specific hormones.

The exact nature of the adaptation induced by recurrent hypoglycemia is uncertain, but data from animal studies suggest it may be due to a hypoglycemia-induced increase in glucose transporter (GLUT 1) activity at the blood-brain barrier.[38-41] This increase in GLUT 1 activity acts to maintain brain glucose uptake during subsequent hypoglycemia. Thus, the central nervous system experiences less neuroglycopenia, and activation of the counterregulatory and autonomic mechanisms is reduced.[21,22] Davis et al[23] have recently proposed that prior increases in plasma cortisol may also contribute to the hypoglycemia-associated reductions in counterregulatory hormone responses. They hypothesized that increased circulating cortisol levels activate the type II glucocorticoid receptor, resulting in downregulation of CRF and ACTH responses and decreased central autonomic activation. The current data would also suggest that central mechanisms operate in the adaptation of the counterregulatory response to recurrent hypoglycemia. However, the relative contribution of these proposed central mechanisms remains to be clarified.

In the current study, the fasting morning ACTH and cortisol levels were significantly reduced following two episodes of interval hypoglycemia but not euglycemia on the previous day. The reduction of basal morning cortisol levels following recurrent hypoglycemia was reported previously by Widom and Simonson[14] and may reflect the negative-feedback effect of cortisol to reduce subsequent ACTH levels rather than a specific effect of hypoglycemia. Nelson and Tindall[42] demonstrated that a morning infusion of ACTH 0.25 mg over 8 hours resulted in significantly lower AM fasting cortisol levels the following day. Previous studies of ACTH infusion as a diagnostic test for adrenal insufficiency[42,43] found that maximum cortisol values achieved with bolus ACTH infusions are inversely related to basal cortisol levels. Although maximum cortisol levels in two sequential 2-hour episodes of hypoglycemia in this study did not reach those achieved with a 4-hour ACTH infusion, the relative cortisol elevation might be sufficient to reduce basal ACTH levels.

A number of studies have suggested that while exposure to recurrent hypoglycemia reduces the response to subsequent hypoglycemia, this adaptation is specific for the hypoglycemic stimulus.[25-28] In a previous study from our group, the cortisol response to an overnight metyrapone test was intact in a group of IDDM subjects in strict glycemic control, despite a low cortisol response to hypoglycemia.[28] Similarly, cortisol responses to exercise remain intact in IDDM subjects following recurrent hypoglycemia.[26-27] The data from the current study also suggest that adaptations in the cortisol response to hypoglycemia are stimulus-specific. However, we cannot rule out the possibility that a failure to match our ACTH stimulus to the submaximal hypoglycemic stimulus to cortisol secretion masked subtle changes in adrenal secretory function. A "low-dose" ACTH stimulation test (1 μg) may have provided greater sensitivity for identification of adrenal adaptation, but it would not have replicated our hypoglycemic stimulus either.[44] ACTH

stimulation tests using doses less than 1 μg would have been required; however, these low doses elicit a wide range of responses in normal subjects[45] and have a maximal 30% variability in an individual,[46] therefore making it difficult to interpret results. Thus, the most clinically relevant standard ACTH stimulation test was used to provide the most reproducible means for elucidating changes in adrenal hormone stores.

Basal ACTH and cortisol and the incremental cortisol response to ACTH are similar on day 3 after hypoglycemia and euglycemia. The most conservative interpretation of these data would suggest that hypoglycemia does not impair the subsequent cortisol response to a standard ACTH stimulation test. Such findings have clinical implications in the treatment of non–insulin-dependent diabetics who require testing of the hypothalamic-pituitary-adrenal axis but have experienced recent or recurrent hypoglycemia. However, when ACTH and cortisol levels on day 1 are compared against those on day 3, basal ACTH and cortisol levels are lower and stimulated incremental cortisol levels and the cortisol AUC are higher. In view of the augmented cortisol response to ACTH following hypoglycemia, it is likely that both the endogenous ACTH release due to hypoglycemia and the exogenous ACTH infusions primed the adrenal gland to augment cortisol responsiveness. Kolanowski et al[47] described this phenomenon using sequential ACTH infusions. ACTH 0.25 mg infused over 8 hours at 9:00 AM on two occasions separated by 0 to 3 days resulted in a significantly increased cortisol response during the second infusion. The potentiation was attributed to upregulation of the enzymes of cortisol synthesis due to ACTH exposure, a mechanism that would be consistent with the time course of the present study.

In conclusion, our results show that exposure to intermittent hypoglycemia in healthy humans reduces fasting morning cortisol, ACTH, and glucagon levels, but increases the cortisol response to a subsequent ACTH infusion. These results suggest that recurrent hypoglycemia may modulate baseline ACTH and cortisol levels via a central neural mechanism. In contrast, the endogenous ACTH produced during hypoglycemia may result in an increased cortisol response to exogenous ACTH infusion. Thus, adrenal function testing with a standard exogenous ACTH infusion to exclude possible adrenal insufficiency is not impaired by prior exposure to hypoglycemia in normal subjects. Moreover, it suggests that the impaired cortisol response in patients with well-controlled IDDM is likely due to adaptation at a central (hypothalamic-pituitary) level rather than decreased adrenal responsiveness.

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