Counterregulatory Hormone and Symptom Responses to Insulin-Induced Hypoglycemia in the Postprandial State in Humans

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Plasma counterregulatory hormones and symptoms were measured during hypoglycemia in the postprandial and in the fasting state in humans to establish differences in physiological responses. We studied 8 nondiabetic subjects and 10 subjects with type 1 diabetes on two different occasions during clamped insulin-induced hypoglycemia (2.4 mmol/l) in the sitting position. On one occasion, subjects ate a standard mixed meal, and on the other they remained fasting. In response to postprandial as compared with fasting hypoglycemia, nondiabetic subjects exhibited lower total symptom scores (6.6 ± 0.4 vs. 11.5 ± 0.8, $P = 0.001$), which was due to less hunger (1.1 ± 0.1 vs. 4.2 ± 0.2), lower suppression of plasma C-peptide (0.23 ± 0.1 vs. 0.08 ± 0.07 nmol/l, $P = 0.032$), and greater responses of plasma glucagon (248 ± 29 vs. 163 ± 25 ng · l^{-1} · min^{-1}, $P = 0.018$), plasma adrenaline (4.5 ± 0.6 vs. 3.1 ± 0.4 nmol · l^{-1} · min^{-1}, $P = 0.037$), norepinephrine (3.8 ± 0.3 vs. 3.2 ± 0.2 nmol · l^{-1} · min^{-1}, $P = 0.037$), and pancreatic polypeptide (217 ± 12 vs. 159 ± 22 pmol · l^{-1} · min^{-1}, $P = 0.08$). Except for plasma C-peptide, responses in diabetic subjects were similarly affected. Notably, in diabetic subjects responses of glucagon, which were absent in the fasting state, nearly normalized after a meal. In conclusion, in the postprandial compared with the fasting hypoglycemic state, total symptoms are less, but counterregulatory hormones are greater and responses of glucagon nearly normalize in type 1 diabetic subjects.
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Hormonal responses to insulin-induced hypoglycemia have generally been studied in the postabsorptive state in the supine position. The physiological principles of counterregulation are well established (1,2).

To the best of our knowledge, no study has so far examined responses of counterregulatory hormone to hypoglycemia induced by insulin after ingestion of a mixed meal in the sitting position in humans. Yet, the question is interesting because responses to insulin hypoglycemia induced in the postprandial state might differ from responses in the fasting state. For example, differences in portal blood glucose might result in different stimulation of liver glucosensors (3). In addition, increase in plasma amino acids after meal ingestion might stimulate glucagon responses more than in the fasting state (4). Likewise, symptoms of hypoglycemia might well differ in the postprandial as compared with the fasting state. Better understanding of the physiology of counterregulatory mechanisms in the postprandial state might be relevant to the problem of postprandial hypoglycemia in diabetic subjects after administration of rapid-acting insulin analogs at meals (5,6).

The present studies were undertaken to establish the differences in physiological responses of counterregulatory hormones, substrates, and symptoms to hypoglycemia in the fasting compared with the postprandial condition in normal nondiabetic subjects and in subjects with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Subjects. Institutional Review Board approval was obtained for these studies. Eight healthy nondiabetic volunteers (five men aged 31 ± 3.1 years, BMI 23 ± 1.3 kg/m^2) were studied. Ten subjects with type 1 diabetes on long-term intensive insulin treatment (10) (6 men aged 29 ± 2.4 years, diabetes duration 12 ± 2.7 years, BMI 22 ± 0.7 kg/m^2, HbA_{1c} 7.2 ± 0.3%) were recruited among those attending the outpatient Diabetes Clinic of the Section of Internal Medicine and Endocrine and Metabolic Sciences, Department of Internal Medicine, University of Perugia. At the time of the study, all type 1 diabetic subjects were free of any detectable microangiopathic complication and were negative at the screening for autonomic neuropathy, as judged on the basis of a standard battery of cardiovascular tests (7).

Design of studies. All nondiabetic and diabetic volunteers were studied on two different occasions at random order, at 2- to 3-week intervals, after giving written informed consent. In diabetic subjects, care was taken to avoid preprandial, postprandial, and nocturnal blood glucose <4.0 mmol/l (72 mg/dl) over the week before studies, as previously reported (8). On the day before studies, patients had their usual insulin treatment with the last subcutaneous NPH insulin injection at ~2300. On the morning of the studies, patients had their usual subcutaneous injection of rapid-acting insulin analog at breakfast (150 g milk, 50 g toasted bread) between 0700 and 0730 and were admitted to the General Clinical Research Center of the Section of Internal Medicine at ~0830 and remained in the sitting position until the end of the studies. A hand vein of the nondominant arm was cannulated retrogradely and maintained in a hot box (~60°C) for sampling of arterialized-venous blood (9). A superficial vein of the ipsilateral arm was also cannulated for infusion of insulin and glucose (further discussed below). The two veins were maintained patent by means of 0.9% NaCl infusion (0.5 ml/min). At 0930, an intravenous infusion of human regular insulin was begun in a feedback fashion to maintain plasma glucose at 5.5 mmol/l (100 mg/dl), as previously described (10), and continued until 1200 (time 0 min). On one occasion, a solid mixed meal (450 Kcal, 46% carbohydrate, 32% lipids, 22% proteins—pasta, meat, and vegetables with 15 g olive oil) was served at 1200 and eaten in 15–20 min. On the other occasion, subjects were kept fasting.

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AUC, area under the curve; FFA, free fatty acid.

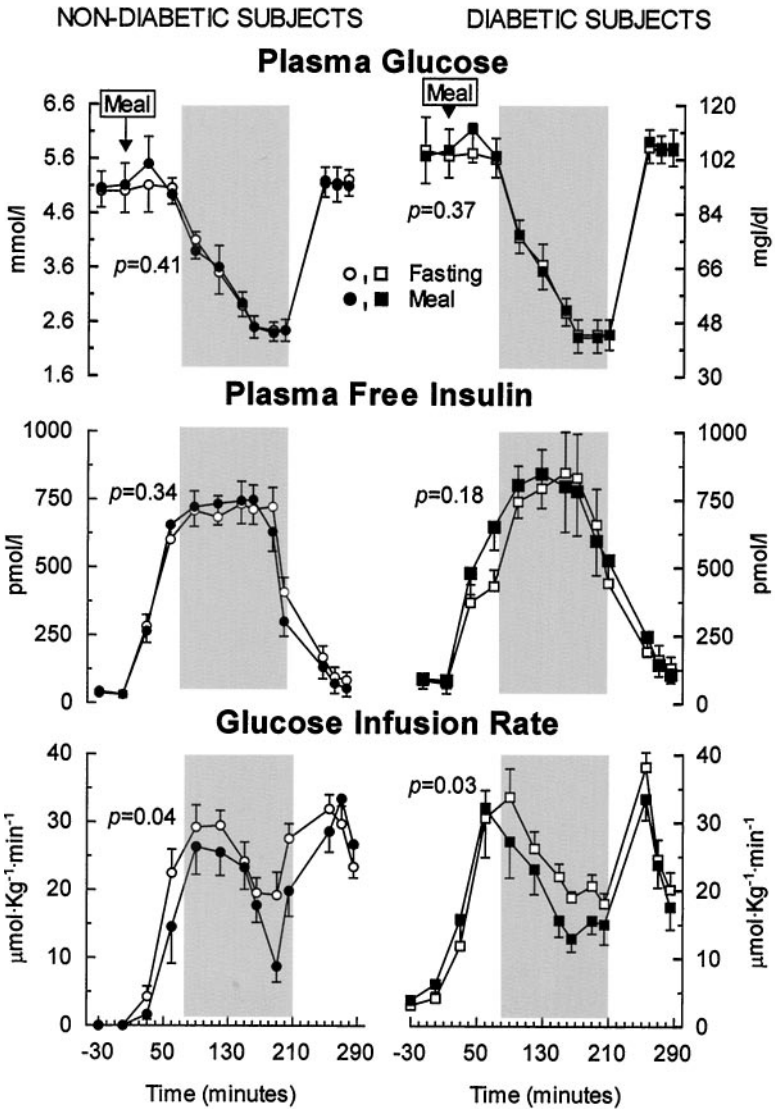

FIG. 1. Plasma glucose and free insulin concentrations and rates of glucose infusion in the fasting and postprandial hypoglycemia studies in normal nondiabetic (circles) and in diabetic (squares) subjects. The stippled areas depict the hypoglycemic sessions (75–205 min) of studies. *P* values indicate study by time interactions from repeated measures ANOVA.

Nondiabetic volunteers were admitted at 0830 on the day of the study after breakfast consumed at home. Afterward, nondiabetic subjects were cannulated as described for type 1 diabetic subjects and studied in an identical manner to type 1 diabetic subjects except no insulin was infused. At 1200 (time 0 min), in both type 1 and nondiabetic subjects, intravenous insulin was infused at the rate of 2 mU · kg^{-1} · min^{-1} till 1525 (time 205 min). Thereafter, the rate of insulin infusion was reduced to 0.5 mU · kg^{-1} · min^{-1} in subjects with type 1 diabetes and withdrawn in nondiabetic subjects. After 1200 (0 min), glucose was infused intravenously at a variable rate to maintain a plasma glucose concentration at 5.5 mmol/l (100 mg/dl) for 75 min. After 75 min, the rate of glucose infusion was decreased to reach the target plasma glucose plateau of 2.4 mmol/l (44 mg/dl) at 165 min in both studies. The hypoglycemic plateau was maintained until 205 min. After 205 min, in both studies the rate of glucose infusion was increased in order to restore euglycemia in 10 min and to maintain euglycemia for 70 min until the end of the studies (285 min).

In all studies, blood was drawn at regular intervals for the measurement of plasma glucose, insulin, counterregulatory hormone, pancreatic polypeptide, and nonglucose substrates. Plasma amino acids were sampled at baseline (−30 and 0 min), during the hypoglycemic plateau (165, 190, and 205 min), and after recovering from hypoglycemia (255, 270, and 285 min).

Analytical methods. Plasma glucose was measured by means of a Beckman glucose analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma insulin, C-peptide, glucagon, growth hormone, cortisol, adrenaline, norepinephrine, glycerol, β-OH-butyrate, lactate, and pancreatic polypeptide were measured by previously described assays (11,12). To remove antibody-bound insulin, plasma was mixed with an equal volume of 30% polyethylene glycol immediately after blood collection in both type 1 diabetic patients and nondiabetic subjects (13). HbA_{1c} was determined by high-performance liquid chromatography using a Hi-AUTO A1C, TM HA 8,121 apparatus (DIC, Kyoto Daiichi, Kogaku, Japan) (values in nondiabetic subjects <6.1%, Diabetes Control and Complications Trial [DCCT] aligned). Plasma free fatty acid (FFA) concentrations were measured using a commercial kit (Wako NEFA C test kit; Wako Chemicals, Neuss, Germany). Plasma amino acids were determined by ion-exchange chromatography (14).

Statistical analysis. All data were subjected to repeated-measures ANOVA with Huynh-Feldt adjustment for nonsphericity (15). Post hoc comparisons (Tukey's test) were performed to pinpoint specific differences on significant interaction means. The areas under the curve (AUCs) of counterrgulatory hormones and substrates at the clamped hypoglycemia period (165–205 min) were calculated according to the trapezoidal rule and analyzed by paired or unpaired Student's *t* test as appropriate. Data in text are given as means ± SE and were considered significantly different at $P < 0.05$. Statistical analysis was carried out using NCSS 2001 software (Kaysville, UT) (16).

RESULTS

Plasma glucose and insulin concentrations and rates of glucose infusion. Both in the fasting and meal studies, plasma glucose was maintained at euglycemia in both nondiabetic and type 1 diabetic subjects for 75 min by variable infusion of glucose (Fig. 1). Thereafter, in both studies the rate of glucose infusion was decreased in order to reduce plasma glucose concentrations to the target hypoglycemic plateau of 2.4 ± 0.08 mmol/l (44 ± 1.4 mg/dl) in nondiabetic subjects and 2.4 ± 0.07 mmol/l (44 ±

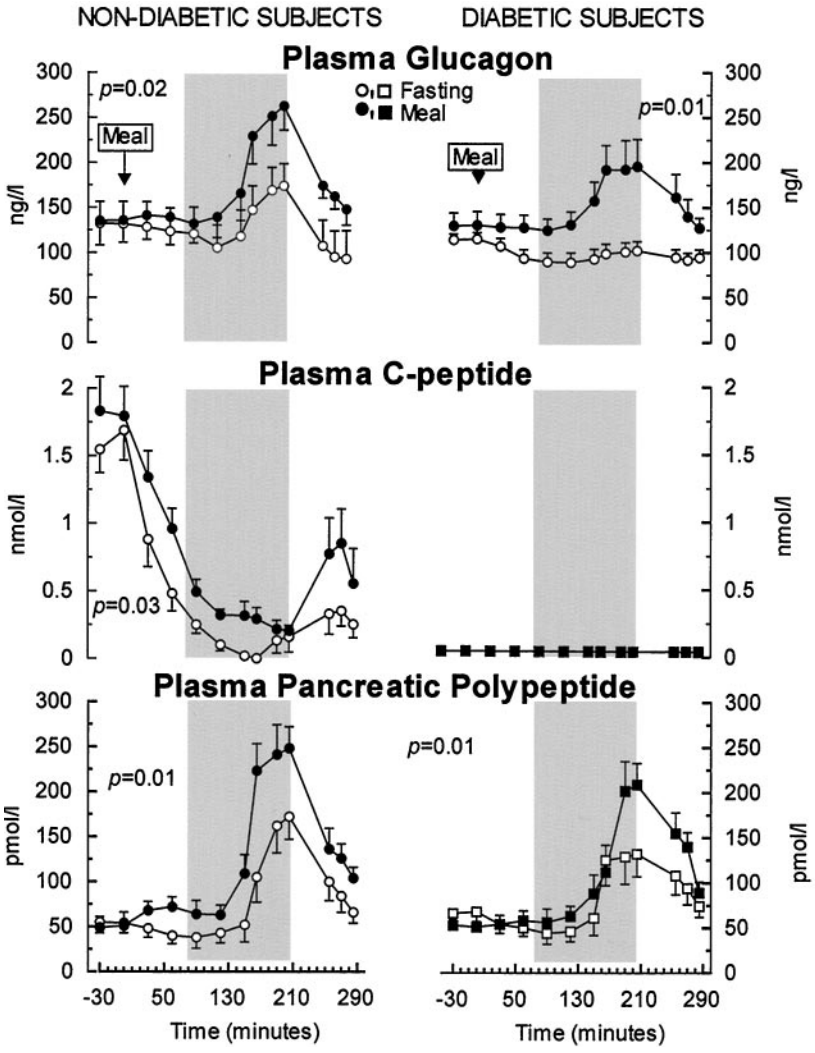

FIG. 2. Plasma glucagon, C-peptide, and pancreatic polypeptide concentrations in the fasting and in the postprandial hypoglycemia studies in normal nondiabetic (circles) and in diabetic (squares) subjects. The stippled areas depict the hypoglycemic sessions (75–205 min) of studies. *P* values indicate study by time interactions from repeated measures ANOVA.

1.2 mg/dl) in diabetic subjects at 165 min. Plasma glucose concentrations were maintained at the plateau of 2.4 ± 0.04 mmol/l (44 ± 0.8 mg/dl) until 205 min; they subsequently increased to 5.5 ± 0.05 mmol/l (99 ± 1 mg/dl) at 215 min and maintained at 5.6 ± 0.03 mmol/l (101 ± 0.6 mg/dl) until the end of the studies with no differences between groups ($P > 0.05$). Plasma glucose concentrations in the fasting and meal studies were not different.

Plasma insulin concentrations did not differ between nondiabetic and diabetic subjects in the fasting and postprandial states. However, baseline plasma insulin concentrations were lower in nondiabetic than in diabetic subjects (30 ± 3 vs. 69 ± 6 pmol/l, respectively, $P < 0.001$) in the fasting study as well as in the meal study (33 ± 4 vs. 72 ± 8 pmol/l, $P < 0.001$).

The rates of glucose infusion ($AUC_{165–205\ min}$) were lower during hypoglycemia plateau in the meal study than during the fasting study in diabetic subjects (14.5 ± 0.6 vs. 19.2 ± 1.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [2.6 ± 0.1 vs. 3.5 ± 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$], respectively, $P = 0.018$) and in nondiabetic subjects (16 ± 2.8 vs. 22.4 ± 2.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [2.9 ± 0.5 vs. 4.1 ± 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$], respectively, $P = 0.04$). However, there were no differences in the glucose infusion rates between nondiabetic and diabetic subjects either before or after the hypoglycemic plateau.

Plasma glucagon, C-peptide, and pancreatic polypeptide concentrations. Plasma glucagon levels were similar at baseline in diabetic and nondiabetic subjects in both the fasting and the meal studies (Fig. 2, Table 1). In the fasting study, plasma glucagon concentrations increased to a peak of 182 ± 26 ng/l ($P = 0.011$ vs. baseline 131 ± 24 ng/l) in nondiabetic subjects, whereas it did not increase in diabetic subjects. In the meal study, the response of glucagon was potentiated in both groups. The area under glucagon curve and the peak response of glucagon both in nondiabetic and diabetic subjects were greater than in the fasting study (Table 1). In diabetic subjects the response of glucagons to postprandial hypoglycemia was lower than that of nondiabetic subjects but greater than that of nondiabetic subjects in the fasting state.

Plasma C-peptide concentrations in nondiabetic subjects decreased less in response to hypoglycemia in the meal study (nadir 0.23 ± 0.1 nmol/l at 190 min) than in the fasting study (nadir 0.08 ± 0.07 nmol/l at 165 min) ($P = 0.032$) and increased more after restoration of euglycemia (0.8 ± 0.2 vs. 0.4 ± 0.1 nmol/l, $P = 0.045$). Mean plasma C-peptide was greater in the meal than in the fasting study in nondiabetic subjects (0.8 ± 0.1 vs. 0.5 ± 0.1 nmol/l, respectively, $P = 0.009$). Plasma C-peptide concentrations were undetectable in diabetic subjects in both studies.

TABLE 1
Plasma counterregulatory hormone and pancreatic polypeptide concentrations

	Nondiabetic subjects			Type 1 diabetic subjects		
	Hypo	Hypo + meal	*P*	Hypo	Hypo + meal	*P*
Glucagon						
AUC (ng · l^{-1} · min^{-1})	163 ± 25	248 ± 29	0.018	100 ± 9	193 ± 25	0.011
C_{max} (ng/l)	182 ± 26	272 ± 28	0.004	118 ± 10	208 ± 30	0.003
Adrenaline						
AUC (nmol · l^{-1} · min^{-1})	3.1 ± 0.4	4.5 ± 0.6	0.037	1.7 ± 0.5	2.9 ± 0.5	0.043
C_{max} (nmol/l)	3.9 ± 0.5	5.1 ± 0.84	0.11	2.2 ± 0.6	3.3 ± 0.82	0.095
Noradrenaline						
AUC (nmol · l^{-1} · min^{-1})	3.2 ± 0.2	3.8 ± 0.3	0.045	2.1 ± 0.3	3.1 ± 0.3	0.005
C_{max} (nmol/l)	3.8 ± 0.29	4.5 ± 0.35	0.037	2.7 ± 0.26	3.6 ± 0.36	0.011
Cortisol						
AUC (μg · dl^{-1} · min^{-1})	16.8 ± 2.4	17 ± 2.2	0.721	15.2 ± 2.3	15.7 ± 2.3	0.117
C_{max} (μg/l)	21 ± 2.2	22 ± 2.4	0.608	16.7 ± 1.8	18.8 ± 2.2	0.093
Growth hormone						
AUC (μg · l^{-1} · min^{-1})	17.8 ± 3.2	11.3 ± 3.7	0.073	26.4 ± 4.2	22 ± 3.5	0.138
C_{max} (μg/l)	35.8 ± 5.5	25.9 ± 4.6	0.062	42 ± 7.0	35 ± 5.5	0.058
Pancreatic polypeptide						
AUC (pmol · l^{-1} · min^{-1})	159 ± 12	217 ± 22	0.008	136 ± 7	200 ± 15	0.001
C_{max} (pmol/l)	189 ± 9	231 ± 24	0.022	148 ± 6	219 ± 6.5	0.010

Data are means ± SE. *P* values calculated from hypo vs. hypo + meal comparisons.

Plasma pancreatic polypeptide concentration in response to hypoglycemia increased more in the postprandial than in the fasting state both in nondiabetic and type 1 diabetic subjects.

Plasma adrenaline and norepinephrine concentrations. Plasma adrenaline responses to hypoglycemia in the fasting state were lower in type 1 diabetic subjects than in nondiabetic subjects ($P = 0.045$) (Fig. 3, Table 1). Plasma adrenaline responses to hypoglycemia were greater in the meal than in the fasting study both in nondiabetic and diabetic subjects. However, responses remained lower in type 1 diabetic subjects than in nondiabetic subjects (Table 1, $P = 0.047$).

Plasma norepinephrine response to fasting hypoglycemia increased less in type 1 diabetic subjects than in nondiabetic subjects (Table 1, $P = 0.018$). After the meal, responses of plasma norepinephrine increased in both groups compared with the fasting study and were no longer different between nondiabetic and diabetic subjects ($P = 0.108$).

Plasma cortisol and growth hormone concentrations. Responses of plasma cortisol to hypoglycemia in nondiabetic and type 1 diabetic subjects were similar both in the fasting and postprandial states (P = NS) (Fig. 4, Table 1). Responses of plasma growth hormone were lower in the postprandial state than in the fasting state, but statistical significance was achieved only in nondiabetic subjects.

Plasma nonglucose substrate and amino acid concentrations. Plasma FFA decreased during hypoglycemia in nondiabetic and diabetic subjects during both fasting and postprandial hypoglycemia (Tables 2 and 3). In fasting hypoglycemia, FFAs were more suppressed in nondiabetic than in diabetic subjects (AUC 46 ± 8 vs. 76 ± 5 μmol · l^{-1} · min^{-1}, $P = 0.039$, respectively). In postprandial hypoglycemia, FFAs were less suppressed than in fasting hypoglycemia until the end of the study in both nondiabetic and diabetic subjects (Table 2).

Plasma glycerol concentrations decreased less during the postprandial than the fasting hypoglycemia in both nondiabetic and diabetic subjects. However, in diabetic subjects, plasma glycerol concentrations remained higher than in nondiabetic subjects in both fasting hypoglycemia (AUC 50 ± 4 vs. 23 ± 3 μmol · l^{-1} · min^{-1}, $P = 0.004$, respectively) and postprandial hypoglycemia (AUC 74 ± 7 vs. 44 ± 5 μmol · l^{-1} · min^{-1}, $P < 0.001$, respectively).

Plasma β-OH-butyrate concentrations were suppressed during hypoglycemia in the fasting state to a similar extent in both nondiabetic and diabetic subjects. However, by the end of the study the posthypoglycemic increase was greater in the diabetic than in the nondiabetic subjects. In response to postprandial hypoglycemia, plasma β-OH-butyrate concentrations were less suppressed than in the fasting state in both nondiabetic and type 1 diabetic subjects. In the latter, the posthypoglycemic increase was nearly threefold greater than in the fasting state.

Plasma lactate increased in response to hypoglycemia in the fasting state in both nondiabetic and type 1 diabetic subjects, although the increase in the former was greater than the latter (AUC 1.7 ± 0.1 vs. 1.4 ± 0.1 μmol · l^{-1} · min^{-1}, $P = 0.048$, respectively). In response to hypoglycemia after a meal, plasma lactate increased more than in the fasting state in both nondiabetic and type 1 diabetic subjects with no difference between groups (AUC 2.0 ± 0.1 vs. 1.8 ± 0.2 μmol · l^{-1} · min^{-1}, $P = 0.383$, respectively).

Branched (valine, leucine, and isoleucine) and nonbranched chain amino acid (BCAA and N-BCAA, respectively) concentrations were similar at baseline in nondiabetic and diabetic subjects in both the fasting and postprandial hypoglycemia studies (Table 3). In response to fasting hypoglycemia, both BCAA and N-BCAA decreased to a similar extent in normal nondiabetic subjects and in diabetic patients. In contrast, when hypoglycemia was induced in the postprandial state, BCAA and N-BCAA concentrations increased as compared with baseline and remained increased to the end of study with no differences between nondiabetic and type 1 diabetic subjects.

Symptoms. The score of responses of autonomic but not neuroglycopenic symptoms to fasting hypoglycemia was

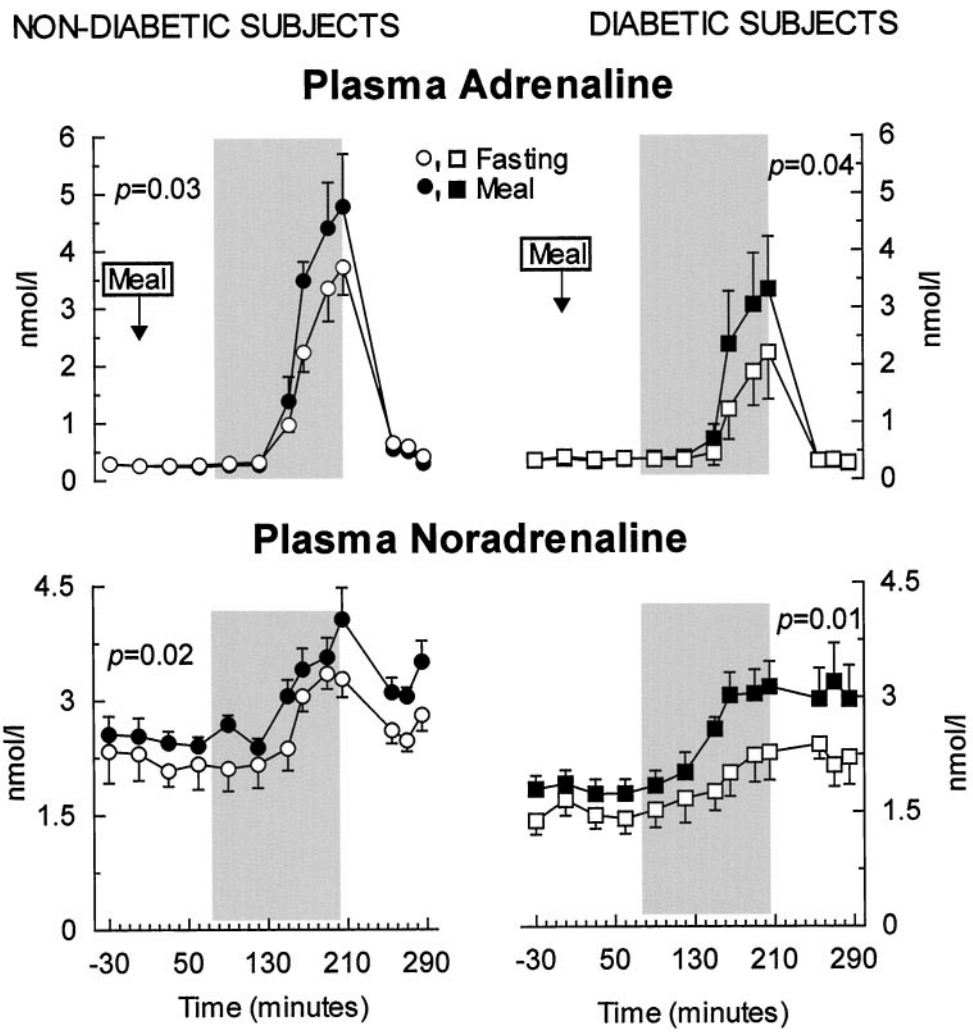

FIG. 3. Plasma adrenaline and norepinephrine concentrations in the fasting and in the postprandial hypoglycemia studies in normal nondiabetic (circles) and in diabetic (squares) subjects. The stippled areas depict the hypoglycemic sessions (75–205 min) of studies. *P* values indicate study by time interactions from repeated measures ANOVA.

lower in diabetic than in nondiabetic subjects (5.3 ± 1.0 vs. 9.3 ± 1.1, $P = 0.018$) (Fig. 5). Overall, the score of autonomic symptoms increased less during postprandial than fasting hypoglycemia in nondiabetic subjects (5.1 ± 0.5 vs. 9.3 ± 1.1, $P = 0.008$) and tended to be lower in diabetic subjects (3.8 ± 0.5 vs. 5.3 ± 1.0, $P = 0.082$). However, the result was entirely attributable to the single symptom, "hunger," which decreased from 4.2 ± 0.2 to 1.1 ± 0.1 (fasting and meal, respectively) in nondiabetic subjects and from 2.5 ± 0.1 to 1.0 ± 0.1 in diabetic subjects. The remaining autonomic symptoms were unchanged. The neuroglycopenic symptom scores were not statistically different in fasting compared with postprandial hypoglycemia in both nondiabetic (1.5 vs. 2.1, $P = 0.212$) and diabetic (1.6 vs. 2.2, $P = 0.224$) subjects.

DISCUSSION

The present studies were undertaken to establish the differences in physiological responses to insulin-induced hypoglycemia in the postprandial compared with the fasting state in humans. Both nondiabetic and type 1 diabetic subjects were studied. The results indicate that in normal nondiabetic subjects, the postprandial compared with the fasting state, first affects the responses of hormones of both A and B cells of pancreatic islets, as shown by the lower suppression of insulin secretion and the potentiation of glucagon response, then potentiates the responses of the rapid-acting counterregulatory hormone adrenaline in the postprandial state, and finally reduces the responses of symptoms (primarily autonomic) in the postprandial compared with the fasting state. The effect of a meal on the responses in diabetic and nondiabetic subjects was qualitatively similar, although some quantitative differences in responses to hypoglycemia between nondiabetic and diabetic subjects remain. Of note, in type 1 diabetic subjects the responses of glucagon, which were absent in the fasting state, nearly normalized after a meal. Taken together, these results highlight the important differences of physiological responses to hypoglycemia in the fed compared with the fasting state in both nondiabetic and diabetic subjects. To the best of our knowledge, these are new findings.

In the present studies the postprandial state had important effects on physiological responses of hormones produced by A- and B-cells of pancreatic islets in response to an insulin-induced decrease of plasma glucose.

Suppression of endogenous insulin secretion—the first line of defense in the prevention of hypoglycemia (2)—is less in the postprandial than in the fasting state despite similar ambient plasma glucose and insulin concentrations. Interestingly, this occurred not only during the progressive decrease of plasma glucose to the hypoglyce-

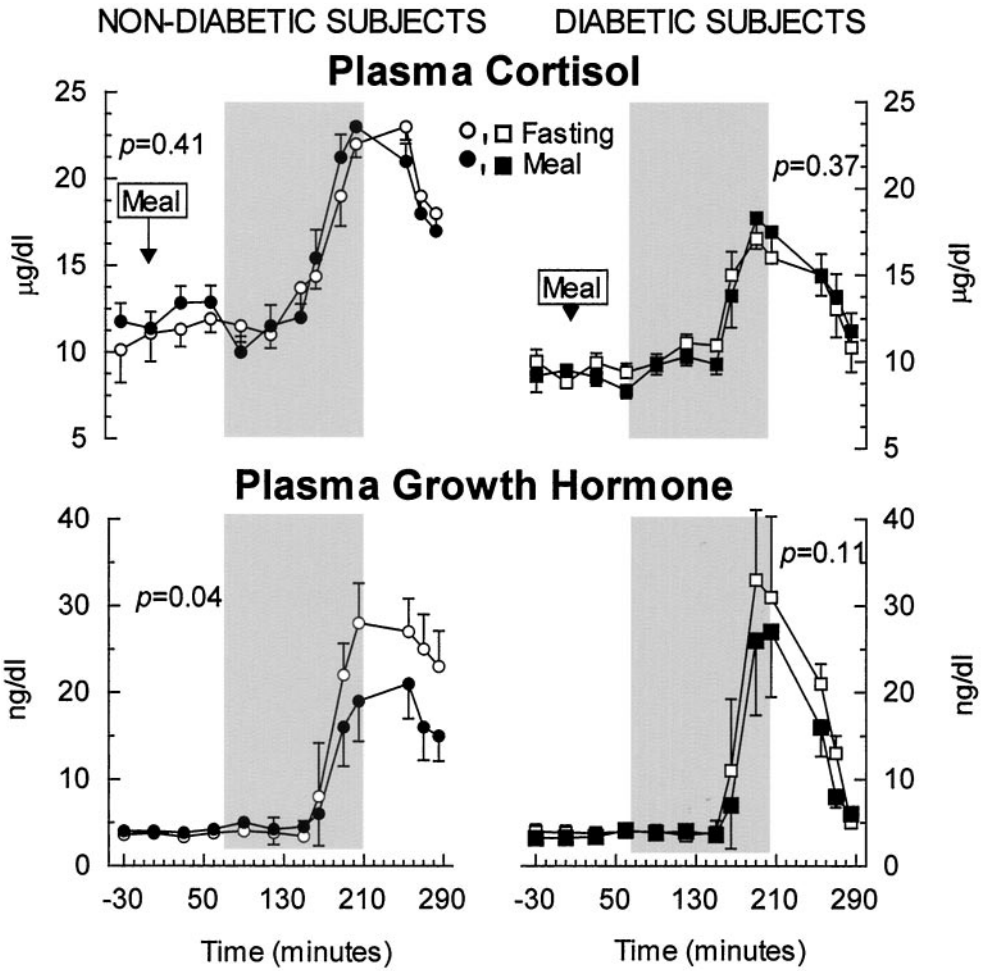

FIG. 4. Plasma cortisol and growth hormone concentrations in the fasting and postprandial hypoglycemia studies in normal nondiabetic (circles) and in diabetic (squares) subjects. The stippled areas depict the hypoglycemic sessions (75–205 min) of studies. *P* values indicate study by time interactions from repeated measures ANOVA.

mia plateau, but also during its recovery to euglycemia. This may be explained by the "incretine" effects of a meal as well as increased parasymphatetic activiy, as indicated by an increase in plasma pancreatic polypeptide (Fig. 2) (17).

In the present experiments, the suppression of endogenous insulin secretion during hypoglycemia is due initially to exogenous insulin infusion as well as to a subsequent decrease in plasma glucose. Although a control experiment in euglycemia was not performed in the present studies, failure of endogenous insulin secretion to respond appropriately to recovery to hypoglycemia and to decrease in plasma insulin in the late part of both fasting and meal studies (after 205 min) should most likely be attributed to inhibitory effects by intra-islet norepinephrine and circulating catecholamines, primarily adrenaline (18). The fact that endogenous insulin secretion in the late euglycemic part of meal experiments increased only modestly compared with the fasting study speaks in favor of a marked inhibitory effect by antecedent greater adrenaline response in the postprandial compared with the fasting study, first reported by Frier at al. (19), despite stimulation of α-cell secretion by meal amino acids. Clearly, such a sophisticated mechanism of regulation of endogenous insulin secretion is not present in type 1 diabetic patients and is a major counterregulatory defect in response to hypoglycemia (2)

In addition to the effects on B-cell of pancreatic islet, the postprandial state exerted important effects on pancreatic A-cell response to hypoglycemia as well. In normal nondiabetic subjects the postprandial state prevented the initial suppression of glucagon by insulin and almost doubled the subsequent glucagon response to hypoglycemia. Notably, this occurred despite less suppression of intra-islet insulin in the postprandial compared with the fasting state (18,20). Among the several components of the meal used in the present studies, amino acids (4,21–24), glucose (24,25), and free fatty acids all may have potentiated the pancreatic islet A-cell response to hypoglycemia. Of note, the elevation of amino acids in plasma in the postprandial state of the present studies was in the order of 0.2–0.6 mmol/l (Table 3), i.e., in the range reported after a mixed meal (26). Although the absolute plasma glucagon responses were lower than in nondiabetic subjects, glucagon responses in type 1 diabetic subjects were superimposable on those of nondiabetic subjects in the fasting state.

The above observations point out that pancreatic islet A-cell of type 1 diabetic subjects, which loses responses to hypoglycemia shortly after clinical onset of diabetes (27), does maintain responses to nonglucose stimuli, as first described by Gerich et al. (4). Indeed, the present study shows that in type 1 diabetic subjects the postprandial state exerts a permissive effect on responses of plasma glucagon to hypoglycemia. The results of the present study indicate that the recovered responses of glucagon to hypoglycemia in the postprandial state in type 1 diabetes

TABLE 2
Plasma nonglucose substrate concentrations

	Nominal plasma glucose (mg/dl)													
	100	100	100	100	90	71	52	44	44	44	100	100	100	
Time (min)	−30	0	30	60	90	120	150	165	190	205	255	270	285	*P*
Free fatty acids (μmol/l)														
Nondiabetic subjects														
Hypo	349 ± 51	347 ± 50	277 ± 45	98 ± 12	83 ± 14	44 ± 7*	37 ± 6*	41 ± 8*	46 ± 8*	50 ± 8*	58 ± 9*	61 ± 7*	72 ± 7*	0.009
Hypo + meal	367 ± 46	364 ± 57	265 ± 36	117 ± 17	92 ± 14	82 ± 13	77 ± 12	89 ± 10	104 ± 24	147 ± 26	135 ± 22	131 ± 27	140 ± 29	
Diabetic subjects														
Hypo	360 ± 36	357 ± 36	282 ± 20	168 ± 6	116 ± 5*	44 ± 4*	45 ± 5	59 ± 6*	65 ± 5*	79 ± 5*	88 ± 6*	112 ± 18*	117 ± 12*	0.011
Hypo + meal	312 ± 53	304 ± 47	252 ± 12	190 ± 6	161 ± 9	85 ± 4	74 ± 9	122 ± 18	126 ± 16	130 ± 4	132 ± 5	166 ± 20	206 ± 35	
Glycerol (μmol/l)														
Nondiabetic subjects														
Hypo	63 ± 4	68 ± 6	55 ± 6	36 ± 7*	21 ± 4*	17 ± 3*	16 ± 3*	15 ± 2*	22 ± 2*	31 ± 5*	44 ± 6	75 ± 4	93 ± 9	0.018
Hypo + meal	67 ± 4	62 ± 5	57 ± 4	51 ± 5	41 ± 6	40 ± 6	40 ± 6	39 ± 6	44 ± 6	47 ± 5	53 ± 5	84 ± 13	98 ± 14	
Diabetic subjects														
Hypo	72 ± 8	76 ± 9	65 ± 5	54 ± 4	52 ± 4	46 ± 4	44 ± 6	41 ± 3*	52 ± 4*	55 ± 6*	69 ± 5*	75 ± 5*	81 ± 5*	0.023
Hypo + meal	77 ± 9	72 ± 10	60 ± 4	56 ± 4	53 ± 3	47 ± 2	49 ± 2	66 ± 2	75 ± 10	80 ± 12	95 ± 12	95 ± 12	106 ± 14	
β-Hydroxybutyrate (μmol/l)														
Nondiabetic subjects														
Hypo	298 ± 103	307 ± 71	295 ± 71	163 ± 66	89 ± 44	45 ± 17	27 ± 9	21 ± 7*	33 ± 7*	33 ± 7*	37 ± 11*	38 ± 15*	48 ± 16*	0.041
Hypo + meal	337 ± 66	324 ± 54	226 ± 31	140 ± 22	99 ± 19	93 ± 8	102 ± 10	101 ± 3	126 ± 6	165 ± 20	133 ± 13	112 ± 13	117 ± 19	
Diabetic subjects														
Hypo	196 ± 16	187 ± 24	144 ± 23	78 ± 19	61 ± 17	43 ± 9	45 ± 11	53 ± 11	63 ± 12*	65 ± 16*	71 ± 16*	76 ± 17*	160 ± 23*	0.012
Hypo + meal	211 ± 20	202 ± 20	149 ± 12	105 ± 12	72 ± 16	53 ± 12	53 ± 12	50 ± 11	155 ± 19	208 ± 22	269 ± 9	352 ± 26	451 ± 54	
Lactate (μmol/l)														
Nondiabetic subjects														
Hypo	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1*	1.1 ± 0.1*	1.0 ± 0.1*	1.2 ± 0.1	1.4 ± 0.1	1.9 ± 0.2*	1.9 ± 0.2*	1.6 ± 0.2*	1.5 ± 0.2	1.4 ± 0.2	0.009
Hypo + meal	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	2.1 ± 0.2	2.3 ± 0.2	1.9 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	
Diabetic subjects														
Hypo	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.2*	1.5 ± 0.2*	1.5 ± 0.1*	1.3 ± 0.2*	1.2 ± 0.1	1.2 ± 0.1	0.039
Hypo + meal	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.7 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	1.4 ± 0.2	1.4 ± 0.2	

Data are means ± SE. *$P < 0.05$ vs. hypo + meal.

TABLE 3
Plasma branched and nonbranched chain amino acid concentrations

	Baseline	Hypo	Recovery	*P*
BCAA (mmol/l)				
Nondiabetic subjects				
Hypo	0.37 ± 0.02	0.23 ± 0.02*	0.26 ± 0.02*	0.008
Hypo + meal	0.35 ± 0.02	0.55 ± 0.03	0.41 ± 0.03	
Diabetic subjects				
Hypo	0.36 ± 0.02	0.21 ± 0.02*	0.25 ± 0.02*	0.002
Hypo + meal	0.37 ± 0.01	0.52 ± 0.03	0.43 ± 0.03	
N-BCAA (mmol/l)				
Nondiabetic subjects				
Hypo	1.32 ± 0.08	1.07 ± 0.06*	1.12 ± 0.07*	0.023
Hypo + meal	1.28 ± 0.08	1.57 ± 0.07	1.5 ± 0.06	
Diabetic subjects				
Hypo	1.24 ± 0.07	1.00 ± 0.06*	1.08 ± 0.07*	0.011
Hypo + meal	1.30 ± 0.07	1.60 ± 0.08	1.41 ± 0.07	

Data are means ± SE. *$P < 0.05$ vs. hypo + meal.

are not simply driven but rather modulated by meal because responses of glucagon become evident only when plasma glucose decreases below a threshold and wanes during recovery of plasma glucose back to euglycemia. Thus, it is primarily plasma glucose, which controls pancreatic A-cell responses to glucose in the setting of the permissive effect of postprandial condition. Notably, the recovered response of glucagon to hypoglycemia in type 1 diabetic subjects occurred despite hyperinsulinemia and lack of physiological decrease in intra-islet insulin concentration in nondiabetic subjects in whom it was less in the postprandial state. Theoretically, resuscitation of glucagon responses to hypoglycemia in type 1 diabetic subjects opens possibilities to improve the prognosis of severe hypoglycemia in affected subjects. Amino acids, which increased in plasma by ~30% in the present studies after a mixed meal, are likely to account for most, if not all, of the responses. In the only study in which the possible stimulatory effects of amino acids on responses of plasma glucagon to hypoglycemia have been studied in type 1 diabetes marginal, if any, effects have been reported (28). However, in that study amino acids were infused intravenously after the onset of hypoglycemia, whereas in the present study amino acids were increased in plasma before the induction of hypoglycemia. It is thus possible that "sensitization"of the pancreatic islet A-cell with amino acids before hypoglycemia is required in type 1 diabetic subjects in order to restore responses of glucagon to the decrease in plasma glucose. A qualitatively similar finding has been reported by Wiethop and Cryer (29) after alanine administration at bedtime in subjects with type 1 diabetic subjects, which protects against nocturnal hypoglycemia. The same authors have reported stimulation of glucagon by alanine in the absence of hypoglycemia (23).

In the present studies the responses of pancreatic

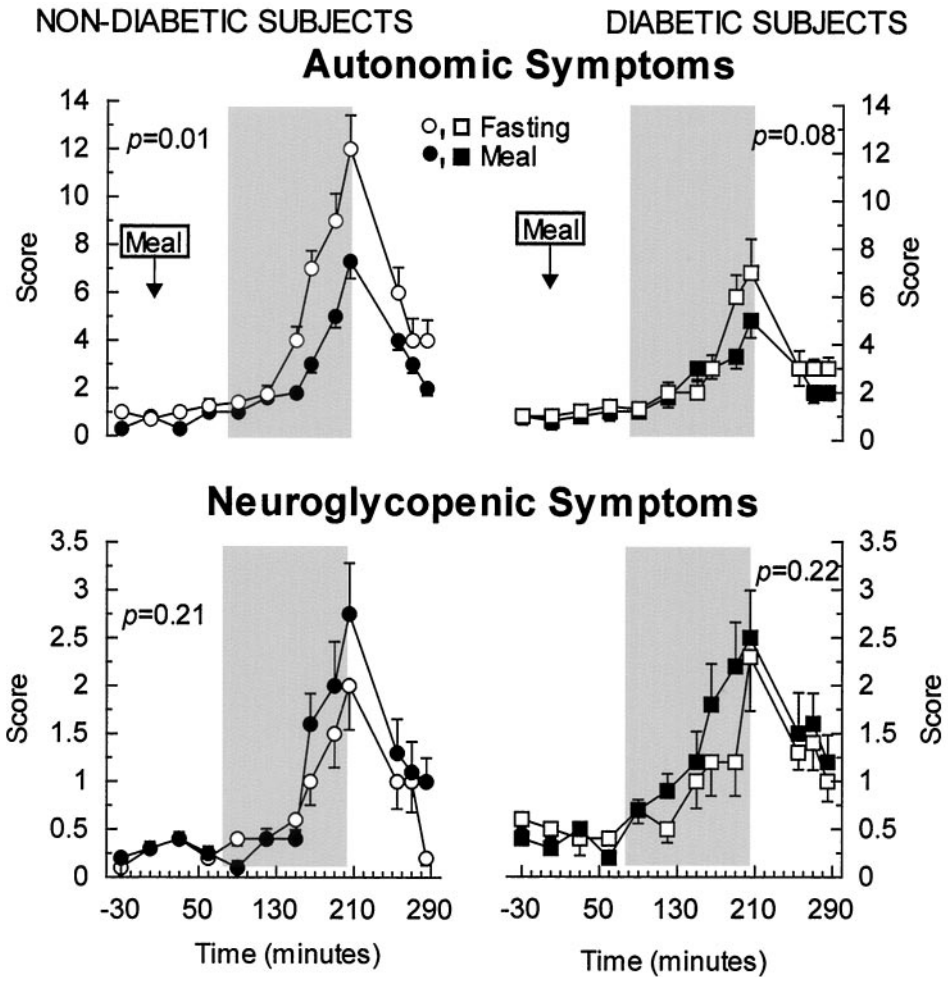

FIG. 5. Autonomic and neuroglycopenic symptom scores in the fasting and postprandial hypoglycemia studies in normal nondiabetic (circles) and in diabetic (squares) subjects. The stippled areas depict the hypoglycemic sessions (75–205 min) of studies. *P* values indicate study by time interactions from repeated measures ANOVA.

polypeptides were also increased in the postprandial state in both normal nondiabetic and type 1 diabetic subjects. This is likely due to the early neural stimulation of a meal on pancreatic polypeptide secretion (30). Although it is well known that plasma concentrations of pancreatic polypeptide increase after a meal (31) as well as after hypoglycemia (32), to the best of our knowledge, the present observation of greater increase in response to hypoglycemia in the postprandial compared with the fasting state is a new finding.

In the present studies, the postprandial condition resulted in potentiation of plasma adrenaline and norepinephrine responses to hypoglycemia compared with the fasting state in both nondiabetic and diabetic subjects to a similar extent. Notably, the increase in norepinephrine is underestimated by its increased clearance in the postprandial state (33). While the importance of increased responses of adrenaline in terms of defense against hypoglycemia is well known (34), the mechanisms of the increased responses during postprandial hypoglycemia in the present studies remain to be determined. Hypotheses include a generalized activation of the sympathetic nervous system in the postprandial compared with the fasting state due to baroflex activation and stimulation of hepato-portal glucose sensors by portal hyperglycemia after meal ingestion, as previously observed in dogs (3). In this regard, the results of the present studies, obtained with a mixed meal before inducing hypoglycemia, are closer to the finding in humans by Heptulla et al. (24) than those of Smith et al. (35) who have both given glucose orally. Although teleologically it would make little sense for nature to potentiate hormonal counterregulatory responses to hypoglycemia in the postprandial compared with the fasting state, it is possible that hepato-portal hyperglycemia offsets the hepato-portal glucose sensors. Under these conditions, glucose sensors within the brain (36) might reinforce their secretory signals to both pancreatic islets and adrenal medulla and sympathetic nerve endings.

Responses of plasma cortisol in the postprandial and fasting state were similar in nondiabetic and diabetic subjects. Responses of growth hormone were reduced in the postprandial state in nondiabetic subjects, most likely as result of increase in plasma FFAs (Table 4) (37).

Taken together, these results indicate that the responses of counterregulatory hormones to hypoglycemia are greater in the postprandial than in the fasting sate. The lower responses of symptoms to hypoglycemia induced in the fasting state in diabetic compared with nondiabetic subjects indicate that diabetic subjects of the present studies suffered to some extent from hypoglycemia unawareness. The responses of symptoms to hypoglycemia were reduced in the postprandial compared with the fasting state in both nondiabetic and diabetic subjects. However, the effect was largely evident for autonomic symptoms but not for neuroglycopenic symptoms (Fig. 5), and was largely, if not exclusively, due to abolition of hunger. Taken together, the results indicate that with the exception of the autonomic symptom hunger, the responses of symptoms to postprandial hypoglycemia do not differ from those in the fasting state. Thus, the greater plasma adrenaline and norepinephrine responses to hypoglycemia in the postprandial state do not translate into greater autonomic symptoms.

In the present studies, after 60–70 min the postprandial condition resulted in a larger availability of nonglucose substrates amino acids, FFAs, glycerol, β-hydroxybutyrate, and lactate, compared with in the fasting state, which may have been relevant as gluconeogenetic substrates for endogenous glucose output. Although β-hydroxybutyrate, amino acids, and lactate might have, at least in part, served as fuel for the brain (38), in the present studies responses of counterregulatory hormones glucagon and adrenaline were potentiated, and those of symptoms were not reduced with the exception of hunger (discussed previously). Taken together, these findings indicate that in the present studies the brain's use of nonglucose substrates was marginal, if any, because one would expect counterregulatory hormone response to be reduced and not potentiated in the postprandial compared with the fasting state.

In conclusion, the present study indicates relevant differences in the physiology of responses to hypoglycemia in the postprandial compared with the fasting state in humans. These differences are common to both nondiabetic and type 1 diabetic subjects. Responses of hormones produced by A- and B-cell pancreatic islets are affected, with less suppression of endogenous insulin secretion and greater stimulation of glucagon; responses of plasma adrenaline, norepinephrine and pancreatic polypeptide are potentiated. The responses of symptoms are not affected with the notable exception of hunger, which is markedly reduced in the postprandial hypoglycemia. As expected, in the postprandial state there is greater availability of nonglucose substrates. Thus, the postprandial state affords greater defenses to hypoglycemia compared with fasting not only due to absorption of oral glucose but also because of greater counterregulatory hormone responses. The most relevant result of the present study is the recovery of glucagon responses to postprandial compared with fasting hypoglycemia in type 1 diabetes. Additional studies are needed to fully explore the potential of this finding as well as to establish the relative role of nutrients, e.g., carbohydrate versus proteins versus lipids, in modifying responses to hypoglycemia in the postprandial compared with the fasting state.

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