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Multiple defects in counterregulation of hypoglycemia in modestly advanced type 2 diabetes mellitus

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Abstract

In type 2 diabetes mellitus (T2DM), little is known about hormonal responses to hypoglycemia. In particular, beta-cell responses to hypoglycemia have not been carefully investigated and potentially because of confounding factors or insufficient power, conflicting data have been obtained regarding growth hormone responses. We therefore compared hormonal responses including rates of insulin secretion during a 2-hour hyperinsulinemic hypoglycemic clamp in a relatively large number of nondiabetic (n = 21) and moderately insulin-deficient subjects with T2DM (homeostasis model assessment of beta-cell function [HOMA-%B], 751 ± 160 vs 1144 ± 83 [pmol/L]/[mmol/L], $P < .04$) (n = 14) matched for age, sex, and body mass index. Subjects with T2DM were excluded for antecedent hypoglycemia, and baseline glycemia was controlled by a variable infusion of insulin overnight. Although both groups of subjects had indistinguishable plasma glucose levels at baseline and virtually identical levels of plasma insulin and glucose throughout the hypoglycemic clamp, insulin secretion decreased more slowly in the subjects with T2DM. The time required for insulin secretion to decline to half its baseline level was markedly increased (38.9 ± 4.9 vs 22.3 ± 1.3 minutes [SD], $P < .01$), and insulin secretion decreased to a lesser extent (−0.79 ± 0.17 vs −1.51 ± 0.09 [pmol/L]/kg per minute, $P < .002$). Moreover, responses of glucagon (28.3 ± 7.3 vs 52.8 ± 7.0 ng/L, $P < .05$) and growth hormone (2.9 ± 0.8 vs 6.3 ± 0.9 ng/mL, $P < .04$) were reduced in the subjects with T2DM, whereas responses of epinephrine, norepinephrine, and cortisol were similar to those in nondiabetic subjects (all $P > 0.6$). We conclude that multiple defects exist in hormonal responses to hypoglycemia in T2DM with moderate beta-cell failure. These include delayed and reduced decreases in insulin secretion, and impaired increases of plasma glucagon and growth hormone.
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1. Background

Hypoglycemia impairs cerebral function and may be fatal. It is therefore not surprising that an elaborate system has evolved for its prevention and correction. In humans, this normally involves a decrease in insulin secretion and an increase in the release of several counterregulatory hormones. The decrease of insulin secretion stands high in the hierarchy of counterregulatory factors [1] as indicated by the finding that recovery from hypoglycemia was approximately 50% reduced when, compared with control experiments, peripheral plasma insulin concentrations were about 2-fold increased by an infusion of insulin despite equivalent portal insulin levels [2]. Among the counterregulatory hormones, glucagon is considered to be most important [1]. Epinephrine, although normally not critical for the defense against hypoglycemia, becomes critical when glucagon secretion is deficient [1]. Cortisol and growth hormone are usually less important than glucagon and epinephrine, but become progressively important for counterregulation as the duration of the hypoglycemic episode increases [1].

Numerous studies have delineated the defects in hormonal responses to hypoglycemia in type 1 diabetes mellitus, but relatively little is known about the hormonal responses in type 2 diabetes mellitus (T2DM). Hitherto evidence indicates that glucagon responses deteriorate with progression of beta-cell dysfunction [3] and that responses of epinephrine and cortisol are usually normal or even increased [3-13], but at least those of epinephrine may become impaired by recent

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antecedent episodes of hypoglycemia as they do in type 1 diabetes mellitus [3]. However, to our knowledge, beta-cell responses to hypoglycemia have not been carefully investigated in T2DM, and inconsistent results have been found regarding the responses of growth hormone [4-13].

Type 2 diabetes mellitus is characterized by a blunted first-phase insulin response to an increase in plasma glucose concentrations [14] and an impaired ability of beta cells to detect and respond to oscillation in plasma glucose [15]. In addition, recent studies indicate that insulin secretion decreases less rapidly during falling blood glucose levels after hyperglycemia in subjects with impaired glucose tolerance [16]. These observations therefore suggest that the decrease in insulin secretion during the development of hypoglycemia may be delayed in T2DM.

With regard to growth hormone, its secretion is diminished by several factors including aging and obesity [17]. Moreover, recent antecedent hypoglycemia has been found to reduce growth hormone responses during subsequent hypoglycemia in some [18,19], but not all studies [20]. However, in most reports on counterregulatory growth hormone responses in T2DM, subjects with T2DM were older and/or more obese than nondiabetic subjects [4-6,8], and in none of them were recent antecedent hypoglycemia listed as an exclusion criterion [4-13]. This may explain why growth hormone responses were found to be reduced in subjects with T2DM in some studies [4-8,13]. On the other hand, plasma free fatty acids (FFAs) are often increased in subjects with T2DM compared with nondiabetic subjects during insulin-induced hypoglycemia [4,9] so that growth hormone responses may be reduced due to the inhibitory effects of FFA on growth hormone secretion [21].

The present studies were therefore undertaken to test the hypotheses that during hypoglycemia, beta-cell and growth hormone responses are abnormal in T2DM. To avoid the limitations of previous studies, we studied a relatively large number of nondiabetic and subjects with T2DM well matched for demographic characteristics and excluded those who had recent antecedent hypoglycemia. Moreover, for proper comparison of beta-cell responses, subjects with T2DM received an infusion of insulin overnight before the hypoglycemic experiment so that baseline plasma glucose concentrations would be virtually identical to those of nondiabetic subjects.

2. Research design and methods

2.1. Subjects

Informed written consent was obtained from 37 subjects, 14 with T2DM (10 men and 4 women, HbA_{1c} 7.4 ± 0.4%) and 23 nondiabetic volunteers (14 men and 9 women) after the protocol had been approved by the Institutional Review Board of the University of Rochester and the Carl T. Hayden VA Medical Center in Phoenix. Diabetic and nondiabetic subjects were matched for age (48.0 ± 1.3 vs 45.5 ± 1.5 years, P = .26), weight (91.7 ± 4.5 vs 90.0 ± 2.8 kg, P = .75), and body mass index (31.4 ± 1.4 vs 31.4 ± 1.2 kg/m^2, P = .99). The diabetic subjects' mean diabetes duration was 5.3 ± 1.3 years, and none had clinical or laboratory evidence of coronary artery disease, nephropathy, proliferative retinopathy, or autonomic neuropathy. All diabetic subjects were being treated with oral hypoglycemic agents including a sulfonylurea, metformin, or a thiazolidinedione, or a combination of these. In addition, 4 subjects were also being treated with NPH insulin at bedtime. Oral hypoglycemic agents and NPH insulin were withdrawn 4 days and 1 day before the experiment, respectively. Nondiabetic subjects had normal fasting glucose tolerance according to American Diabetes Association criteria [22] and no family history of diabetes.

2.2. Protocol

All subjects were admitted to the University of Rochester General Clinical Research Center (n = 17; 4 T2DM, 13 nondiabetic subjects) or the Clinical Research Center of the Phoenix Carl T. Hayden VA Medical Center (n = 20; 10 T2DM, 10 nondiabetic subjects) between 5:00 and 6:00 PM the evening before experiments. Subjects received a standard dinner (41.84 kJ/kg [10 kcal/kg]: 50% carbohydrate, 35% fat, 15% protein) between 6:30 and 7:00 PM and fasted thereafter except for water ad libitum until the experiments were completed. In the diabetic subjects, an antecubital vein was cannulated at approximately 10:00 PM, and a variable intravenous insulin infusion was started to restore normoglycemia overnight [23]. During this period, blood glucose concentrations were measured at 30- to 60-minute intervals and levels less than 90 mg/dL (5.0 mmol/L) were avoided. Normoglycemia was achieved by approximately 3:00 AM in all subjects. The insulin

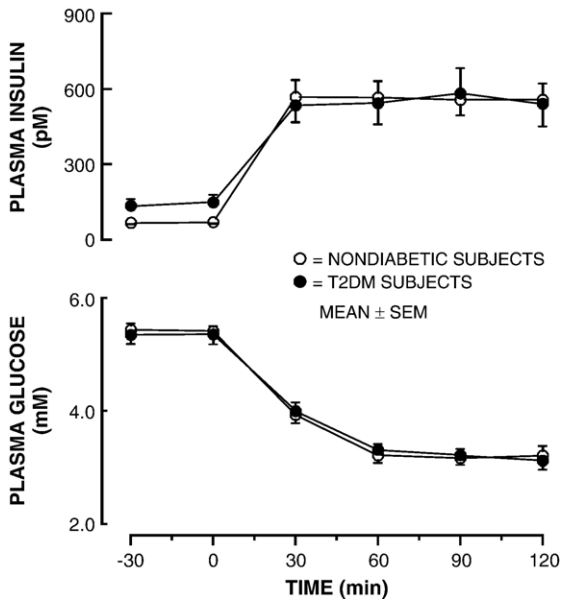

Fig. 1. Plasma concentrations of insulin and glucose in nondiabetic and subjects with T2DM during hypoglycemia.

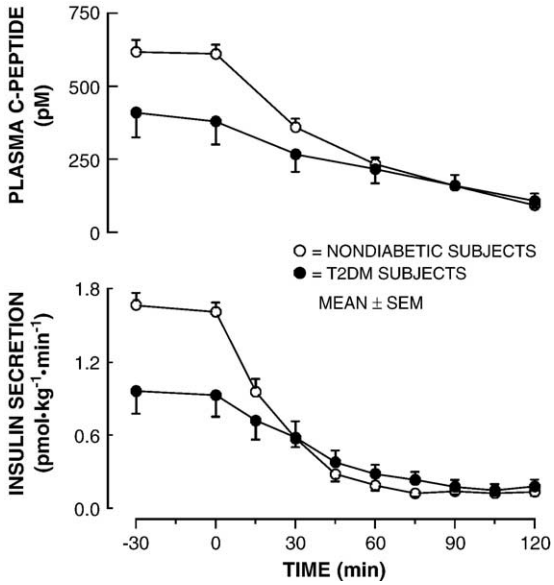

Fig. 2. Plasma C-peptide concentrations and rates of insulin secretion in nondiabetic and subjects with T2DM during hypoglycemia.

infusion that maintained normoglycemia thereafter averaged 1.1 ± 0.2 U/h.

At approximately 7:00 AM, a retrograde venous catheter was inserted into a dorsal hand vein kept in a thermoregulated Plexiglass box at 65°C for sampling arterialized venous blood [24]. At least 1 hour later, blood samples were collected at 30-minute intervals from −30 to 120 minutes for measurement of plasma glucose, insulin, C-peptide, glucagon, epinephrine, norepinephrine, growth hormone, cortisol, and FFAs. At 0 minute, a continuous infusion of insulin (~1.0 mU/kg per minute) was begun, and plasma glucose concentrations were allowed to decrease to 45 to 50 mg/dL (2.5-2.8 mmol/L) where they maintained until 120 minutes using the glucose clamp technique [25]. Some of the subjects (4 T2DM, 7 nondiabetic subjects) received a 90- to 120-minute infusion of normal saline (30 mL/h) before the hypoglycemic clamp as part of a different protocol [26,27]. In these subjects, the last 30 minutes of the saline infusion was considered baseline.

2.3. Analytic procedures

Plasma glucose was determined with a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin, C-peptide, glucagon, growth hormone, and cortisol concentrations were determined by standard radioimmunoassays, and plasma epinephrine and norepinephrine concentrations were measured by a radioenzymatic method as previously described [28]. Plasma FFAs were measured by an enzymatic calorimetric method (NEFA C, Wako Pure Chemical Industries, Osaka, Japan).

2.4. Calculations

Beta-cell function was assessed by using homeostasis model assessment of beta-cell function (HOMA-%B), which was calculated as C-peptide (pmol/L) × 3.33/[glucose (mmol/L) − 3.5] using the average of fasting plasma concentrations. C-peptide was used in place of the originally proposed plasma insulin because of the exogenous infusion of insulin [29]. Rates of insulin secretion were calculated by deconvolution analysis of plasma C-peptide using an open 2-compartmental model [30,31] and population based transition coefficients [32] as described by Hovorka and Jones [33]. The software (ISEC, version 2) was kindly provided by Dr R Hovorka, Center for Measurement and Information in Medicine, City University, London, UK. Hormonal responses to hypoglycemia were determined as the difference between plasma hormonal concentrations during the last 30 minutes of the hypoglycemic clamp and those at baseline. Fractional rate constants defining the decrease in insulin secretion over time were determined by fitting a monoexponential equation to data for insulin secretion using the simulation, analysis, and modeling program (WinSAAM, version 3.0.7). Half-times for insulin secretion were calculated from the fractional rate for decline of insulin secretion.

2.5. Statistical analyses

Unless stated otherwise, data are expressed as means ± SEM. Data of the diabetic subjects were compared with those of the nondiabetic subjects using unpaired 2-tailed Student *t* tests whereby *P* values were adjusted using the Bonferroni procedure [34]. Glucagon responses to hypoglycemia were not normally distributed and rank-transformed before applying the *t* test. Least square linear regression was used to assess correlations. A *P* value of less than .05 was considered statistically significant.

3. Results

3.1. Plasma glucose, insulin, C-peptide, and rates of insulin secretion

After stabilization of normoglycemia overnight by a variable infusion of insulin, baseline plasma glucose concentrations in subjects with T2DM were indistinguishable from those of nondiabetic subjects (5.3 ± 0.2 vs 5.3 ±

Table 1
Counterregulatory responses (decrements and increments from baseline) in nondiabetic subjects and subjects with T2DM

	Nondiabetic subjects (n = 23)	Subjects with T2DM (n = 14)	*P*
Insulin secretion (pmol/kg per minute)	−1.51 ± 0.09	−0.79 ± 0.17	<.002
Plasma glucagon (ng/L)	52.8 ± 7.0	28.3 ± 7.3	<.05
Plasma epinephrine (pmol/L)	1433 ± 277	1516 ± 263	>.8
Plasma norepinephrine (pmol/L)	584 ± 104	497 ± 140	>.6
Plasma growth hormone (μg/L)	6.3 ± 0.9	2.9 ± 0.8	<.04
Plasma cortisol (nmol/L)	290 ± 37	296 ± 40	>.9

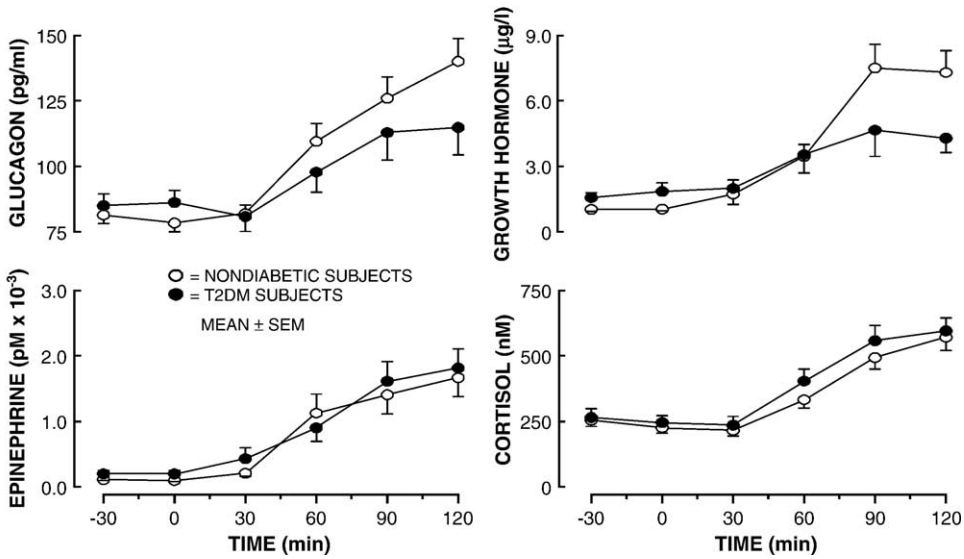

Fig. 3. Plasma concentrations of glucagon, epinephrine, cortisol, and growth hormone in nondiabetic and subjects with T2DM during hypoglycemia.

0.1 mmol/L). Subsequently, during the constant insulin infusion, plasma glucose decreased in a similar fashion in both groups averaging virtually identical levels during the last 30 minutes of the hypoglycemic clamp (both 3.2 ± 0.1 mmol/L) (Fig. 1). The glucose infusion rates required to maintain the clamp during the last 30 minutes were not significantly different between the diabetic and nondiabetic subjects (3.8 ± 1.4 vs 5.6 ± 1.4 μmol/kg per minute, $P > .6$).

Arterial plasma insulin concentrations were greater in subjects with T2DM than in nondiabetic subjects at baseline as a result of the overnight insulin infusion, but were comparable in both groups during the constant insulin infusion (561 ± 94 vs 556 ± 63 pmol/L, $P > .9$) (Fig. 1). Baseline plasma C-peptide (382 ± 80 vs 610 ± 36 pmol/L, $P < .012$), HOMA-%B (751 ± 160 vs 1144 ± 83 [pmol/L]/[mmol/L], $P < .04$), and rates of insulin secretion (0.94 ± 0.18 vs 1.64 ± 0.08 pmol/kg per minute, $P < .002$) were significantly lower in subjects with T2DM than in nondiabetic subjects, indicating moderately impaired beta-cell function (Fig. 2). During the induction of hypoglycemia, plasma C-peptide and rates of insulin secretion decreased more slowly in the subjects with T2DM. Monoexponential curves fitted to data for insulin secretion indicated that insulin secretion in nondiabetic subjects declined to half of the baseline value by 22.3 ± 1.3 minutes (SD), whereas for the subjects with T2DM, 38.9 ± 4.9 minutes (SD) were required for insulin secretion to decrease to half the initial value ($P < .01$). At the end of the hypoglycemic clamp, plasma C-peptide (133 ± 30 vs 127 ± 8 pmol/L) and rates of insulin secretion (0.16 ± 0.04 vs 0.13 ± 0.03 pmol/kg per minute) were however comparable in both groups (both $P > .5$). Nevertheless, because of lower plasma C-peptide and insulin secretion at baseline, decrements of these parameters in subjects with T2DM were reduced (both $P < .002$) (Table 1).

At baseline, plasma concentrations of epinephrine (201 ± 50 vs 105 ± 10 pmol/L, $P < .04$) and growth hormone (1.7 ± 0.3 vs 1.0 ± 0.1 μg/L, $P < .02$) were slightly greater in subjects with T2DM, whereas plasma glucagon (85 ± 5 vs 80 ± 3 ng/L), norepinephrine (976 ± 110 vs 907 ± 133 pmol/L), and cortisol (281 ± 40 vs 238 ± 20 nmol/L) were not significantly different between both groups (all $P > .5$). During hypoglycemia, responses of glucagon were ~45% reduced in subjects with T2DM ($P < .05$), which correlated significantly with their reduced decrement in insulin secretion ($r = 0.58$, $P < .05$). Furthermore, responses of growth hormone were ~50% reduced in subjects with T2DM ($P < .04$), whereas those of epinephrine, norepinephrine, and cortisol were comparable in both groups (Fig. 3, Table 1).

Baseline plasma FFA concentrations were similar in both groups (553 ± 54 vs 582 ± 31 μmol/L, $P > .6$). During the hypoglycemic clamp, plasma FFA decreased to a lesser extent in diabetic subjects so that their plasma FFA levels were approximately 1.5-fold greater than in nondiabetic subjects during the last 30 minutes of the experiment (323 ± 36 vs 205 ± 23 μmol/L, $P < .01$) (Fig. 4).

When data of all subjects were analyzed together, plasma FFA correlated inversely with the percentage of decrement in insulin secretion during the early part of the hypoglycemic

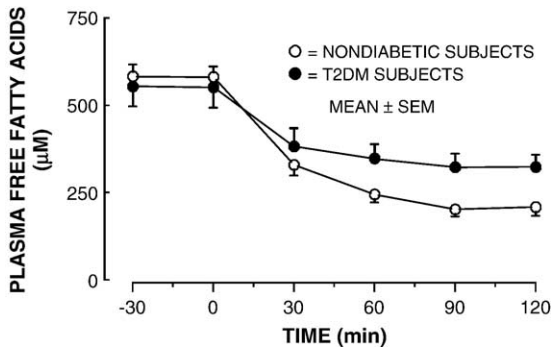

Fig. 4. Plasma concentrations of FFAs in nondiabetic and subjects with T2DM during hypoglycemia.

clamp ($r = -0.41$ at 30 minutes, $P < .01$); moreover, during the last 30 minutes of the hypoglycemic clamp, plasma FFA correlated inversely with growth hormone responses ($r = -0.37$, $P < .03$).

4. Discussion

The present studies demonstrate that 3 of the major glucose counterregulatory mechanisms are abnormal in patients with T2DM who have moderate beta-cell failure. In addition to the reduced glucagon responses found in previous studies [3,9] and confirmed in the present study, insulin secretion decreased less rapidly and to a lesser extent, and growth hormone responses were markedly impaired in the subjects with T2DM. These abnormalities occurred despite virtually identical plasma glucose levels before and during insulin-induced hypoglycemia in both groups.

Our finding that subjects with T2DM had a reduced decrement in insulin secretion during hypoglycemia was not unexpected as insulin secretion is impaired at baseline in T2DM and is nearly completely inhibited during hypoglycemia [13]. The finding that the time required for insulin secretion to decrease to half its baseline value was nearly 2-fold increased (~39 vs 22 minutes) in the diabetic subjects is however novel. As noted in the Background section, Introduction, T2DM is characterized by a blunted first-phase insulin response to an increase in plasma glucose concentrations and an impaired ability of beta cells to detect and respond to oscillation in plasma glucose [14,15]. Moreover, in subjects with impaired glucose tolerance, insulin secretion has been found to decrease less rapidly during falling blood glucose levels after hyperglycemia [16]. These observations suggest that a defect in the feedback loop that couples glucose concentrations to insulin secretion may be responsible for the delayed beta-cell response to hypoglycemia in T2DM.

Additional possible explanations for the diabetic subjects' delayed beta-cell response may be increased stimulation or decreased inhibition of insulin secretion by factors other than glucose, including FFAs, the parasympathetic nervous system (stimulatory), and the sympathetic nervous system (inhibitory). Differences in responses of the autonomic nervous system were probably not involved because subjects were free of autonomic neuropathy and both groups of subjects had equivalent counterregulatory responses of epinephrine and norepinephrine in the present study. In addition, responses of pancreatic polypeptide, an index of the local parasympathetic activity, have previously been found to be normal in similar subjects with T2DM [5].

Boden et al [35] have shown that immediate lowering of plasma FFA from ~600 to ~100 μmol/L by nicotinic acid reduced basal insulin secretion approximately 30% in nondiabetic and subjects with T2DM. Consistent with previous reports [4,9], we found that plasma FFAs were approximately 1.5-fold greater during hypoglycemia in the diabetic subjects than in the nondiabetic subjects despite similar levels at baseline. Accordingly, if plasma FFA were similarly important for insulin secretion under the present experimental conditions as they were under the conditions of the study by Boden et al [35], the diabetic subjects' reduced decrement in plasma FFA might have been partially responsible for the delayed decrease in insulin secretion. This notion is supported by our finding that in the early part of the hypoglycemic clamp, the percentage of decrement in insulin secretion correlated inversely with plasma FFA concentrations.

With regard to growth hormone, we found that its responses to hypoglycemia were more than 50% reduced in subjects with T2DM compared with nondiabetic subjects. Because groups of subjects were well matched for age, sex, and obesity, and subjects with T2DM were excluded for recent antecedent hypoglycemia, these findings provide for the first time clear evidence for impaired counterregulatory growth hormone responses in T2DM independent of these potential confounders.

One important factor for this defect might have yet again been the diabetic subjects' increased plasma FFA levels during hypoglycemia. Increases in plasma FFA levels decrease growth hormone responses to a variety of pharmacologic and physiologic stimuli, including arginine, growth hormone–releasing hormone, and exercise in humans [36-38]. Moreover, decreases in plasma FFA levels by acipimox have been found to increase human growth hormone responses to hypoglycemia [39]. And finally, although not proving cause and effect, plasma FFA correlated inversely with counterregulatory growth hormone responses in the present study.

In agreement with previous studies [3,9], we found that in subjects with T2DM with moderate insulin deficiency, glucagon responses to hypoglycemia were approximately 45% reduced and that these reduced responses correlated with the reduced decrement in insulin secretion during induction of hypoglycemia. These findings are consistent with evidence that a decrease in intraislet insulin, in concert with a decrease in plasma glucose, is normally a signal to glucagon secretion and that a reduced intraislet insulin signal may well explain the reduced glucagon response in insulin-deficient diabetes [26,40].

Despite several defects in hormonal responses to hypoglycemia in the subjects with T2DM, we found that the glucose infusion rates required to maintain the hypoglycemic clamp were, if anything, lower in the diabetic than in the nondiabetic subjects. It is of note that this finding does not imply that counterregulatory glucose kinetics remained unaffected in the subjects with T2DM because no euglycemic control experiments were performed to account for their presumably greater insulin resistance.

In conclusion, our data demonstrate that multiple defects exist in hormonal responses to hypoglycemia in subjects with T2DM who have moderate beta-cell failure. These include decreased responses of glucagon and growth hormone, and delayed and reduced decreases in insulin secretion. Further studies are needed to determine the physiologic and clinical importance of these abnormalities in this population.

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