Insulin Secretion in Type 1 Diabetes

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Type 1 diabetes, a chronic autoimmune disease, causes destruction of insulin-producing β-cells over a period of years. Although many markers of the autoimmune process have been described, none can convincingly predict the rate of disease progression. Moreover, there is relatively little information about changes in insulin secretion in individuals with type 1 diabetes over time. Previous studies document C-peptide at a limited number of time points, often after a nonphysiologic stimulus, and under non–steady-state conditions. Such methods do not provide qualitative information and may not reflect physiologic responses. We have studied qualitative and quantitative insulin secretion to a 4-h mixed meal in 41 patients with newly diagnosed type 1 diabetes and followed the course of this response for 24 months in 20 patients. Newly diagnosed diabetic patients had an average total insulin secretion in response to a mixed meal that was 52% of that in nondiabetic control subjects, considerably higher than has been described previously. In diabetic patients there was a decline of β-cell function at an average rate of 756 ± 132 pmol/month to a final value of 28 ± 8.4% of initial levels after 2 years. There was a significant correlation between the total insulin secretory response and control of glucose, measured by HbA_{1c} ($P = 0.003$). Two persistent patterns of insulin response were seen depending on the peak insulin response following the oral meal. Patients with an early insulin response (i.e., within the first 45 min after ingestion) to a mixed meal, which was also seen in 37 of 38 nondiabetic control subjects, had a significantly accelerated loss of insulin secretion, as compared with those in whom the insulin response occurred after this time ($P < 0.05$), and significantly greater insulin secretory responses at 18 and 24 months ($P < 0.02$). These results, which are the first qualitative studies of insulin secretion in type 1 diabetes, indicate that the physiologic metabolic response is greater at diagnosis than has previously been appreciated, and that the qualitative insulin secretory response is an important determinant of the rate of metabolic decompensation from autoimmune destruction. *Diabetes* 53: 426–433, 2004

Type 1 diabetes is thought to result from the autoimmune destruction of the insulin-producing pancreatic β-cells. The autoimmune response may begin years before the clinical disease onset (1). Clinical presentation occurs at a threshold of impaired β-cell function associated with decreased β-cell mass. A study of the pathologic anatomy of the pancreas from patients soon after clinical presentation suggested that ~10% of normal β-cell mass remains (2). However, studies of C-peptide functional responses to mixed meals have indicated that the remaining insulin response is 33% of normal (2,3). Nonetheless, with time, all individuals with type 1 diabetes are thought to lose insulin production.

Cross-sectional analyses have indicated that there may be sustained β-cell function, particularly in older individuals, years after onset of type 1 diabetes (4–6), but there are very little prospective data concerning the natural history of islet loss, and there are no previous studies utilizing accurate measurements of insulin secretion under physiologic conditions (4,6,7). This information is important for the design of immune intervention studies since a subgroup of patients with an attenuated loss of insulin production may benefit from immune interventions but may also affect the interpretation of studies. There are currently no markers that can identify individuals with a slow versus a more rapid loss of insulin production, but markers of the process may provide insight into its pathogenesis. Indeed, even in the NOD mouse, a widely studied animal model of the disease, there is conflicting information about the loss of β-cell function. Investigators have cautioned that measurements of β-cell mass may be misleading, because there may be increased β-cell proliferation in the prediabetic period, which allows normal β-cell mass to be maintained until it is overwhelmed by the immune attack (8,9).

In humans, the clinical onset of disease is most often abrupt, like in the NOD mouse. Unlike the mouse model, however, patients often enter a clinical remission (i.e., honeymoon) of variable duration, which is thought to be due to improved β-cell function. This β-cell recovery is thought to be due to improved blood glucose control upon commencement of insulin therapy, since hyperglycemia itself may impair insulin response (10–12). Moreover, it was recognized in even the earliest studies that evaluated insulin secretion in type 1 diabetes that improved secretion is associated with better glucose control, raising an

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AUC, area under the curve; ISR, insulin secretory rate; MMTT, mixed-meal tolerance test.

uncertainty about whether the improved glucose control resulted in improved insulin production or vice versa (3).

Insulin and C-peptide are secreted by the pancreas on an equimolar basis, but 50% or more of the active hormone, insulin, is extracted during the first pass through the liver. Hence, measurement of insulin in the peripheral blood does not accurately reflect endogenous insulin secretion. Most studies have approximated insulin secretion with C-peptide levels measured when fasting, at random, or following provocation with mixed meals or glucagon (13–25). While glucagon produces a reliable insulin response, it does not simulate normal physiology while a mixed meal is physiological. Furthermore, a single time point for C-peptide measurement does not provide qualitative information about the insulin response between and within patients over time (3,21–23,25).

While C-peptide has been widely accepted as a useful approximation of insulin secretion under steady-state conditions, its measurement may not correctly reflect insulin secretion under non–steady-state conditions (26,27). C-peptide concentrations do not change proportionally with insulin secretory rates (ISRs), as C-peptide is distributed in intra- and extravascular compartments and its metabolic clearance rate may vary between patients (26–28). A long half-life of C-peptide may particularly skew results over short time intervals, since C-peptide values may remain elevated while insulin secretion has actually fallen. Van Cauter et al. (29) have developed standard kinetic parameters to be used in a two-compartment model to calculate ISRs from measurements of peripheral C-peptide levels (deconvolution of C-peptide) (26). This approach provides both quantitative and qualitative data about endogenous insulin secretion.

We wished to determine the natural history of loss of insulin secretion in type 1 diabetes and to identify metabolic or other predictors of the progression of the disease, and elected to study insulin secretion rates with a 4-h mixed-meal tolerance test in patients with new-onset type 1 diabetes and in healthy control subjects. The results of our analysis show that within 10 weeks from initial diagnosis, patients with type 1 diabetes have a mixed-meal–stimulated response that is ~50% of that seen in nondiabetic control subjects, which is far greater than had been previously thought. In addition, we found that the insulin secretory pattern in response to the meal, rather than age, sex, or autoantibodies, correlates most highly with loss of insulin production over time.

TABLE 1
Clinical characteristics of subjects at study entry

	Sex (M/F)	Anti-GAD65 autoantibody positive (%)	Anti–IA-2 autoantibody positive (%)	Age (years)	Weeks after diagnosis	Insulin dose (units/kg)	BMI (kg/m^2)	Basal C-peptide (pmol/ml)	Peak C-peptide (pmol/ml)
All patients with type 1 diabetes (n = 41)	25/16	85	69	14.8 ± 0.93 (7–30)	5.42 ± .29 (2–10)	0.46 ± 0.04 (0–1.06)	19.7 ± 0.56 (14.4–28.3)	0.23 ± 0.03 (0.03–1.14)	0.60 ± 0.04 (0.15–0.30)
Patients with type 1 diabetes followed for 24 months (n = 20)	9/11	85	65	15.3 ± 1.35 (8–30)	6.65 ± 0.37 (4–10)	0.44 ± 0.05 (0–0.83)	19.9 ± 0.72 (14.8–25.5)	0.25 ± 0.05 (0.09–1.14)	0.61 ± 0.06 (0.26–1.30)
Nondiabetic control subjects (n = 38)	16/22	ND	ND	21.8 ± 0.83 (13–30.6)			23.4 ± 0.46 (17.1–28.9)	0.39 ± 0.02 (0.12–0.97)	1.81 ± 1.11 (0.98–4.64)

Data are means ± SE (range). ND, not determined.

RESEARCH DESIGN AND METHODS

Forty-two patients with new-onset type 1 diabetes were recruited for a randomized control trial of a non-FcR binding anti-CD3 monoclonal antibody (30). The patients were between the ages of 7.5 and 30 years and were enrolled within 10 weeks of diagnosis of type 1 diabetes. One-half of the patients were randomized to an observation group, and all underwent mixed-meal tolerance tests (MMTTs) every 6 months. The other patients were treated with the anti-CD3 mAb, and the effects of this treatment have previously been published (30,31). All patients had at least one detectable autoantibody (anti-GAD65 or anti-ICA512) soon after diagnosis. The patients' diabetes management was in the hands of their personal physicians. All patients received at least three injections of short-acting or intermediate-acting insulin and/or continuous subcutaneous insulin therapy (25%) during the follow-up period. The goal of therapy for all patients was to achieve glucose control near normal without severe hypoglycemia.

In addition, 38 healthy individuals without a personal or family history of diabetes were studied on a single occasion. The clinical characteristics of the

TABLE 2
Insulin responses to mixed meal tolerance test at baseline

	Mean total insulin secretion (AUC)	Mean fasting blood glucose (mg/dl)	Mean ISR peak time (min)	Basal ISR (pmol)	Peak ISR (pmol)
All patients with type 1 diabetes (n = 41)	24,744 ± 1,836 (4,522–52,093)	117 ± 5 (70–208)	39 ± 5.0 (7.5–105)	50 ± 4.0 (5.2–101)	172.7 ± 13.6 (30.3–377.7)
Patients with type 1 diabetes followed for 24 months (n = 20)	25,643 ± 2,778 (9,142–52,093)	124 ± 8 (84–208)	31.5 ± 4.5 (7.5–75)	50.4 ± 4.4 (22.3–85.7)	184 ± 21.3 (47.94–377.7)
Nondiabetic control subjects (n = 38)	47,695 ± 2,069 (26,108–81,537)	86.4 ± 1.2 (75–104)	21.9 ± 1.4 (7.5–45)	95.6 ± 6.1 (29–230)	668 ± 45.6 (305–1,832)

Data are means ± SE (range).

patients and normal control subjects are presented in Table 1. The study was approved by the institutional review boards at Columbia Presbyterian Medical Center, the National Institute of Diabetes and Digestive and Kidney Diseases, the University of Utah, and the University of California at San Francisco. Because of restrictions in enrolling healthy children in research studies, the normal control subjects were, on average, older than the patients with diabetes. There was, however, considerable overlap in ages between the two groups. All patients or their parents provided written informed consent, and written assent was obtained from minors.

Study design. The patients with diabetes were enrolled on average 5.42 ± 0.29 weeks after initial diagnosis, after achieving metabolic stabilization. Every 6 months an MMTT was performed. Of the 21 patients with diabetes who were assigned to the observation group, one became pregnant before the 24th month and did not complete the last MMTT. A second patient was lost to follow-up after 6 months.

For patients with diabetes, the morning and the previous evening's insulin dose were withheld before the MMTT. An indwelling catheter was placed, and samples were collected for fasting glucose and C-peptide as well as HbA_{1c}. Boost (High Protein) was administered orally at a dose of 6 ml/kg over a 5-min period. Blood samples were drawn at 15, 30, 60, 90, 120, 150, 180, 210, and 240 min in order to capture the complete pancreatic response to the meal. Control subjects underwent an MMTT after an overnight fast.

Data analysis. C-peptide levels were measured by radioimmunoassay at the Diabetes Research and Training Center at the University of Chicago (32). The assay's lower limit of detection was 0.03 pmol/ml with intra- and interassay coefficients of variability of 8 and 11%, respectively. HbA_{1c} levels were measured using a DCA 2000 (Bayer, Indianapolis, IN) (normal range 4.5–6.0%). Anti-GADA and anti-ICA512 autoantibodies were measured with radiobinding assays (33).

ISRs were determined by deconvolution of peripheral C-peptide levels using a two-compartment model of C-peptide kinetics (26). The rate constants used to describe an individual's C-peptide kinetics were standard parameters estimated from the C-peptide decay curves of 200 normal, obese, and diabetic adults (29). Parameters used accounted for an individual's age, sex, height, weight, and diabetic status (29,34). The 0-min values were used to derive the basal ISR rates.

The total area under the insulin secretory response curve (AUC) was determined for each study. This is a measure of the total C-peptide or insulin secretion in picomoles. In addition, the time of peak insulin secretion and slope of rise to peak ISR were determined. Where indicated, the total AUC was expressed over time as a fraction of the initial value (i.e., at study enrollment) to be able to compare values between patients. Insulin secretion was considered negligible when C-peptide levels were ≤0.03 pmol/ml.

Statistical analyses were performed with StatView software (SAS Institute). In the case of missing data (two data points), previous values were carried forward. All significance tests were performed at the $\alpha < 0.05$ level and were two-tailed. Correlations were performed by either a regression plot or a Spearman's rank correlation test. Means were compared by Student's t test. All mean values are expressed ± SE.

RESULTS

Qualitative and quantitative insulin and C-peptide responses to a mixed meal in patients with type 1 diabetes and normal control subjects. MMTTs were performed in 42 individuals with type 1 diabetes and 38 healthy control subjects. Of those with type 1 diabetes, 20 did not receive experimental immunotherapy and were prospectively studied for 2 years, during which time MMTTs were performed every 6 months. The clinical characteristics of these patients and the control subjects are shown in Table 1. Restrictions governing the inclusion of healthy children in research studies resulted in the nondiabetic control subjects being older and having a higher BMI than the patients with type 1 diabetes, but there was considerable overlap in these parameters.

Figure 1 shows C-peptide and calculated ISRs during the MMTT. When compared with the C-peptide response, the ISR curve (ISR values plotted over time) more clearly delineated periods of increased and decreased secretion than could be identified by the C-peptide levels alone. Although we found significant qualitative differences in the

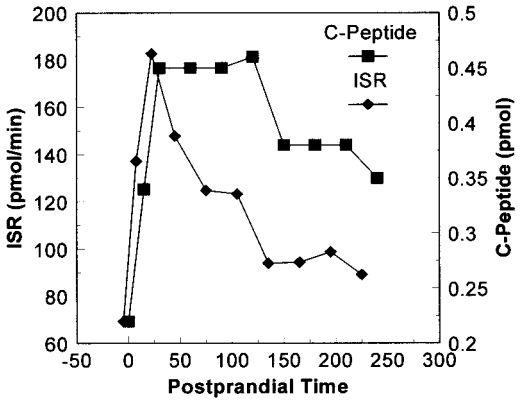

FIG. 1. Insulin secretory responses during the MMTT show qualitative and quantitative changes over time that cannot be appreciated by measurement of C-peptide responses alone. Data are shown from a single representative patient with type 1 diabetes who underwent an MMTT as described in the RESEARCH DESIGN AND METHODS. C-peptide levels were measured by radioimmunoassay and ISRs were calculated as indicated. Because of the half-life of C-peptide and its distribution in two compartments, peripheral C-peptide levels do not necessarily reflect insulin secretion.

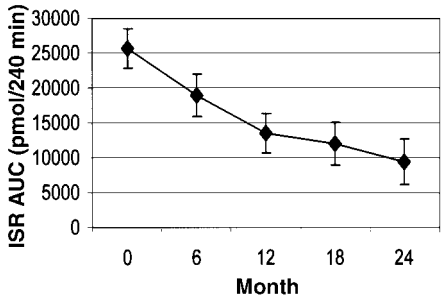

FIG. 2. Changes in insulin secretory responses over 2 years in patients with type 1 diabetes. Subjects with diabetes (n = 20) underwent an MMTT every 6 months for 2 years. The data points represent the group mean (± SE) ISR AUC at each study.

responses described by C-peptide levels and ISRs, there was a strong quantitative correlation between these two analyses (r = 0.973, $P < 0.0001$).

At study entry the mean average insulin secretion total AUC for diabetic patients was 24,744 ± 1,836 pmol (range 4,522–52,093 pmol), which was 51.9% of the response seen in nondiabetic control subjects (Table 2). There was considerable overlap between the responses that were seen in patients with diabetes and normal subjects: 22 of 42 patients with diabetes had a response within the range of control subjects (mean ± 2 SD). The basal insulin response accounted for a greater proportion of the total insulin response in patients with diabetes than in normal control subjects. In addition, the impairment in insulin secretion was more apparent on the peak response than on basal insulin secretion. Whereas the basal insulin secretory rate in patients with diabetes was 52% of the basal insulin secretory rate in nondiabetic control subjects, the peak insulin secretory response in patients with type 1 diabetes was 26% of the peak secretory rate in nondiabetic control subjects. The average time of peak ISR was delayed in the patients with diabetes to 39 ± 5 min ($P < 0.001$), and the patients with diabetes took longer to return to basal levels of ISR over the 240 min of monitoring: 33% of patients with diabetes had not reached basal levels after 240 min, whereas 16% in the normal control group had not reached basal levels by this time. This delay in responsiveness to glucose is similar to responses that have been reported in obese patients with impaired glucose tolerance (35).

Changes in insulin secretory responses over time. The mean total insulin AUC values and the fraction of the starting value were calculated at each point of study (Fig. 2). The slope of the line describing the change in ISR AUC was calculated. On average, there was a loss of insulin secretion over time that averaged 756 ± 132 pmol/month in patients with type 1 diabetes. The average AUC was 70 ± 6.8% (range 25–145%) 6 months after diagnosis, 48 ± 9.1% (range 0–107%) 12 months after diagnosis, 41 ± 8.8% (range 0–103%) 18 months after diagnosis, and 28.3 ± 8.7% (range 0–124%) 24 months after diagnosis, all relative to the response at study entry. All patients had detectable levels of insulin secretion until at least 6 months after study enrollment, but some patients lost detectable C-peptide (i.e., <0.03 pmol/ml) over time: by month 12, 20%, by month 18, 25%, and by month 24, 47% of patients had C-peptide levels that fell below the lower limit of detection (0.03 pmol/ml). Once C-peptide levels were undetectable, secretory responses could not be determined.

Not all patients showed a persistent decline in β-cell function on all of the serial studies. Of 78 follow-up studies, 8 showed ISR AUCs that were greater than tests of the same individual at study entry. The improved studies occurred in 5 of 21 patients (see below).

Relationship between ISRs and glucose control. All patients received clinical care from their endocrinologists and were managed with diet and intensive use of insulin. Approximately 25% of patients used an insulin pump. Consistent with earlier studies, there was a significant correlation between the insulin secretory response and the level of glucose control, reflected by HbA_{1c} (r = 0.401, P = 0.003) (Fig. 3) (3). Similarly, the units of insulin used per day were negatively correlated with insulin total AUC (r = −0.583, $P < 0.008$).

Previous studies have suggested that younger patients (i.e., <12 years) with type 1 diabetes lose their insulin secretory capacity faster than older (i.e., >12 years)

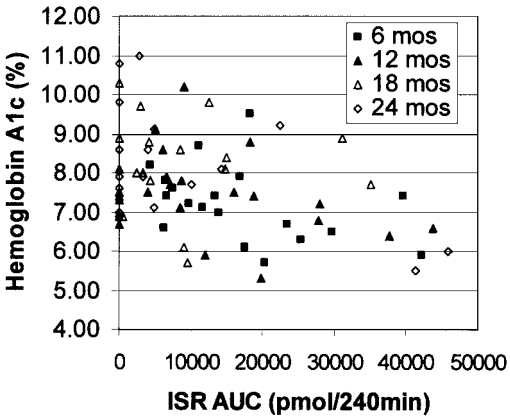

FIG. 3. The quantitative insulin secretory response during the MMTT is inversely related to glucose control, reflected by HbA_{1c} level. The HbA_{1c} and ISR AUC is plotted at each study after study entry in subjects who were prospectively studied over 2 years (at 6, 12, 18, and 24 months). There was a significant inverse correlation between the two measures (r = 0.401, P = 0.003)

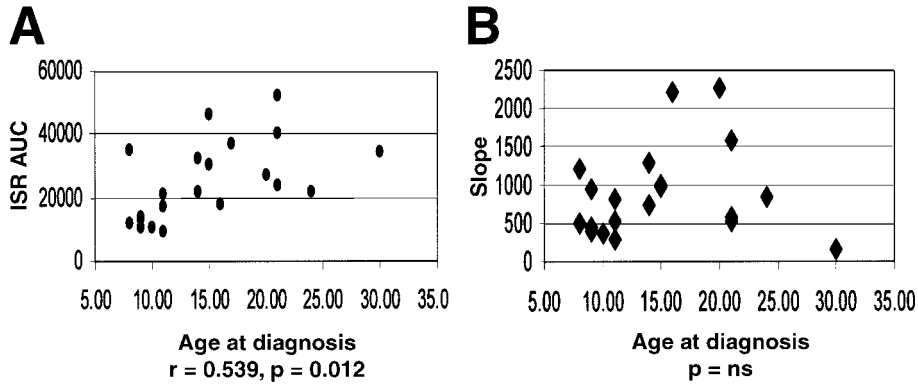

FIG. 4. Insulin secretory reserve is greater at older ages of presentation of type 1 diabetes, but the decline in insulin secretion is not age related. *A*: The insulin secretory response (AUC) during an MMTT at diagnosis is plotted as a function of age of diagnosis in patients who were prospectively followed for 2 years ($r = 0.539$, $P = 0.012$). *B*: The slope describing the change in the insulin secretory response over time was plotted as a function of age. These two indexes were not significantly correlated.

patients. Our analysis showed a positive association between age of diagnosis and the ISR AUC (Fig. 4*A*), but there was no apparent relationship between age and the slope of ISR decline (Fig. 4*B*), or in BMI. The diagnosis of type 1 diabetes had been confirmed by the finding of detectable autoantibodies in all of the patients. We found no relationship between the presence or absence of anti-GAD autoantibodies and the ISR response. We did, however, observe that there was a trend for higher ISR responses in individuals who were ICA512-positive at 0 and 6 months ($P < 0.03$).

Patterns of insulin secretion to a mixed meal in patients with type 1 diabetes and normal control subjects. By calculating insulin secretory rates, we identified two meal-induced insulin secretory patterns (Fig. 5*A* and *B*).

- Early insulin response present: Some individuals demonstrated a rapid increase of ISR, defined as the peak ISR rate occurring within 45 min of the ingestion of the meal, and a slower rate of decline afterward. In some cases there was another peak of insulin production later during the 4-h test, but if the maximal response was before 45 min, they were considered to be in the early-response group.
- Delayed insulin response: Others with type 1 diabetes had a peak response that occurred later, at 45 min or later after consuming the mixed meal, with a more symmetric distribution of ISR before and after the peak. Sixty-two percent (26 of 42) of patients with type 1 diabetes showed an early insulin response, and 38% of patients showed a delayed insulin response. In the normal control subjects, 94% had an early insulin response and 2 of 38 had a delayed response ($P = 0.004$). In patients with diabetes followed for 2 years, 14 had an early and 6 had a delayed insulin response.

The pattern of insulin secretory response observed in the first two studies (at diagnosis and at 6 months) were generally stable over the course of 2 years in the 21 patients who were not drug treated and prospectively studied over 2 years. With time, the reduction in the AUC that occurred was reflected by a decrease in the amplitude of the ISRs, but not by a change in the pattern of the response (Fig. 5*A* and *B*). Two of the patients originally found to have a delayed response on the initial study after diagnosis were later found to have an early response that persisted at all of the follow-up studies and were classified as "early responders" in the analysis. The pattern of insulin response was not associated with BMI, fasting glucose, the

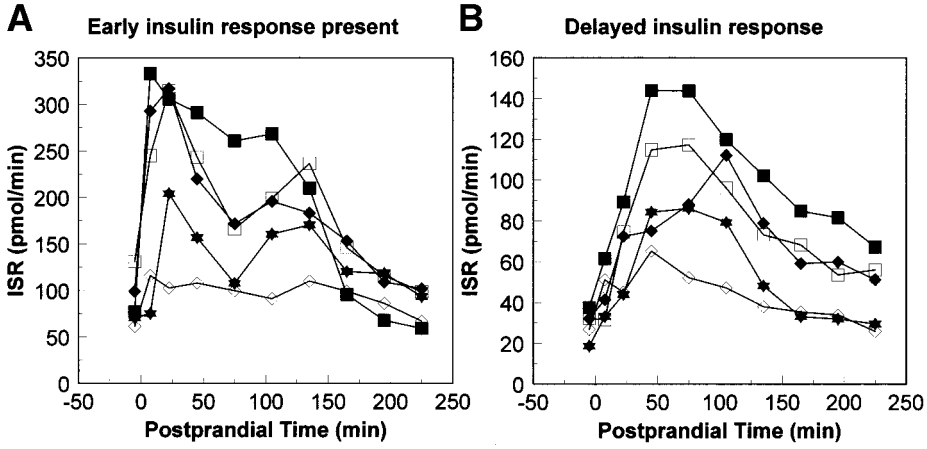

FIG. 5. *A* and *B*: Two patterns of insulin secretion to an MMTT are seen in patients with type 1 diabetes. Two patterns of insulin secretion in response to the mixed meal were observed, based on the presence of an early response to insulin. The data are from two individuals studied every 6 months over 2 years (at study entry [■], 6 months [◆], 12 months [□], 18 months [★], and 24 months [◇])

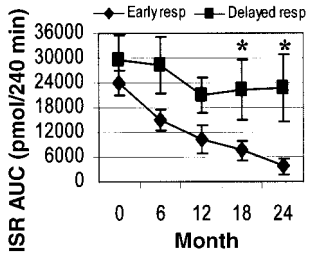

FIG. 6. Patients with an early insulin response during the MMTT have a more rapid decline in insulin secretion over time. The patients who were prospectively studied over 2 years were divided into groups depending on whether the peak insulin secretory response to the mixed meal was early (i.e., within the first 45 min after ingestion, $n = 14$) or late (45 min or later, $n = 6$). The insulin secretory AUC for each group (± SE) is plotted over time. The pattern of secretory response was a significant determinant of the loss of insulin secretion over the 2 years ($P < 0.03$ by repeated-measures ANOVA). *$P < 0.02$.

duration of diabetes, or the absolute AUC response at study entry.

The pattern of insulin secretory response within the first 6 months after diagnosis of diabetes predicts the rate of loss of insulin secretion. The pattern of the insulin response curve predicted the decline of insulin secretion over the 2-year follow-up period ($P < 0.03$ by repeated-measures ANOVA). The decline in insulin secretory response was significantly less in individuals with a delayed insulin response (336 ± 186 pmol/month) compared with those with an early response (936 ± 150, $P < 0.05$) (Fig. 6). At 24 months, patients with an early C-peptide response had total responses that were 14.0 ± 6.4% of the response at study entry, whereas patients with a delayed response had a total response that was 61.8 ± 19.7% of the response at study entry ($P < 0.01$).

DISCUSSION

We have studied insulin responses to the physiologic stimulus of a mixed meal in healthy control subjects and patients with new-onset type 1 diabetes and have followed the changes in these responses over 2 years of diabetes. As such, ours is the first study of insulin secretory dynamics in patients with type 1 diabetes. Although the total insulin secretory response (i.e., the AUC of the ISR) and C-peptide responses were highly correlated, qualitative characteristics of the pancreatic response were not captured by C-peptide values. Therefore, we calculated ISRs during a 4-h MMTT. Our analysis used techniques that were developed by Polonsky and colleagues (29,35,36) using a two-compartment model with standard kinetic parameters. These techniques have previously been used for studies of patients with type 2 diabetes, obesity, and in obese children.

Our data show that patients with type 1 diabetes have a significant insulin secretion reserve at onset of disease: on average, the response to the mixed meal was 52% of the response of nondiabetic control subjects. In fact, over half of subjects with type 1 diabetes had responses that were within the mean ± 2 SD of the responses of the nondiabetic individuals. Previous studies that have reported greater impairment in insulin secretion may have been affected by factors such as glucose toxicity and insulin resistance at the time of diagnosis (8,10,11). In contrast, our patients had all achieved metabolic stabilization before evaluation. Moreover, prior estimates were based on C-peptide measurements in a limited number of subjects. By calculating the ISRs, we were able to fully capture the response, which might otherwise be under- or overestimated by C-peptide levels alone under non–steady-state conditions. Our finding is surprising since it has generally been believed that the residual β-cell mass present at the time of diagnosis of type 1 diabetes is marginal, and in fact, many have questioned the value of interventions at the time of diagnosis because there is little β-cell mass to preserve. However, our findings would indicate that substantial insulin reserve could be maintained with successful interventions at that time. A successful intervention to maintain insulin secretion would have important clinical implications, since the insulin response to a mixed meal was positively associated with HbA_{1c} levels. We believe that the cause of the improved glycemia control was the improved insulin secretion rather than vice versa, as suggested by previous studies (4). We recognize that glucose control per se may affect insulin secretion, although the duration of this effect is not clear (6,37). Other studies have found that the level of insulin production is an important determinant of glucose control. In the Diabetes Control and Complications Trial (DCCT), stimulated C-peptide levels >0.2 pmol/ml were associated with improved HbA_{1c} levels and any detectable insulin reserve was associated with reduced rates of disease complications including hypoglycemia (4,38). The responses we measured to a mixed meal do not necessarily reflect the full insulin secretory reserve that is lost. In fact, our finding of greater impairment of peak compared with basal insulin secretory responses would suggest that a maximal insulin stimulus, such as with a hyperglycemic clamp, may show a greater loss than we have seen. Nonetheless, in the setting of physiologic stimuli, the insulin response in patients at the time of diagnosis of type 1 diabetes is about half of normal levels.

In patients with type 1 diabetes, the response to the MMTT declined, on average, 756 pmol/month. Improvements in responses were uncommon and were not sustained over time. Thus, the appearance of a clinical "honeymoon" more likely reflects a correction of the factors precipitating the presentation of diabetes rather than an actual increase in insulin production, although it is still possible that transient increases (i.e., lasting <6 months after diagnosis) could have occurred but were missed. While all patients had detectable levels of C-peptide even after 6 months, close to half of the subjects had levels of C-peptide that were undetectable by 2 years after diagnosis. It has previously been suggested that older patients with type 1 diabetes (>12 years) have a slower rate of decline in insulin production than younger patients (18,21,23,39). Previous cross-sectional studies, for example from the DCCT, support this notion (4). However, our prospective analysis showed that the slope of the decline is not related to age, at least between the ages of 7 and 30. We did not have the opportunity to study individuals with latent autoimmune diabetes of adults (LADA), but other studies of this group have suggested that the decrease in insulin production in this group is similar to younger patients (40). Instead, we found that the total insulin response at presentation is positively associated with age. The fact that the rate of decline in insulin production is

similar at different ages suggests that factors leading to clinical presentation rather than the natural history of the disease differ between younger and older patients. We also found a relationship between the presence of ICA512 autoantibodies in the first 6 months after diagnosis and decline in insulin secretion. A relationship between autoantibodies and residual insulin production and the natural history of the disease has been described by some, but not all, previous studies (13,17,41). The association that we identified did not, however, predict residual insulin production with longer follow-up.

By analyzing insulin secretory responses in patients with type 1 diabetes, we identified two patterns to the mixed meal. The majority of individuals with diabetes had an early insulin response that reached a peak secretory rate within 45 min of ingesting the meal. This was also the predominant pattern of response in normal control subjects. In other patients the insulin secretory response was delayed, with peak insulin secretory rates occurring at 45 min and later after ingesting the meal. The basis for the different responses is under investigation; the response curve may be affected by factors including gut hormones, protein in the mixed meal, and other secretagogues that may differentially affect subpopulations of β-cells, which are more or less susceptible to immune destruction. The fact that a delayed response is more common in patients with type 1 diabetes than in the normal control subjects suggests it is acquired as part of the disease process, but the pattern of response was generally consistent over a follow-up period of 2 years. This suggests an intrinsic feature of the β-cell population, possibly reflecting differences in subpopulations of β-cells between individuals. Rorsman et al. (42) and Grodsky (43) have shown that the two different response phases of insulin release are mediated by two different populations of secretory granules, and early and later release of insulin are from separate compartments of stored insulin. The striking finding was that there were significant differences in the loss of β-cell function over time between individuals with these two patterns of response—patients with type 1 diabetes who had an early response lost insulin secretory responses more rapidly than those who had a delayed response. The ISR AUCs were not significantly different ($P = 0.47$) at study entry, but the rate of loss of insulin secretion and the fractional AUCs (ISR AUC at 24 months/ISR AUC at study entry) were different after 24 months ($P < 0.03$ and <0.01). One possible explanation for this is that cells with a predominance of early released granules may be more vulnerable to autoimmune attack than those with the late-release granules. Physiologically or functionally different β-cells may express different islet antigens or have different susceptibilities to cell death. Equally likely is that the chronic autoimmune response may lead to "selection" of β-cell subpopulations, characterized by the late response to the mixed meal, that are more resistant to the autoimmune attack. Such cells may be selected during periods of regeneration of β-cells, as has been described in the NOD mouse, before disease presentation (8).

Our finding of differences in disease progression in type 1 diabetes has implications for the design of intervention trials in these patients. It is noteworthy that our evaluation of the 20 patients who were enrolled in our previously reported hOKT3γ1(Ala-Ala) trial had a distribution of insulin responses (13 early, 8 late) that was identical to the untreated subjects from that trial (30). Nonetheless, in trials involving small numbers of subjects, the differences between individuals could have a strong effect on the outcome.

In summary, we have characterized the insulin secretory dynamics of patients with recent onset type 1 diabetes. We found that there is considerably greater insulin secretory capacity at the time of diagnosis of type 1 diabetes than had been previously appreciated. By virtue of the association between ISRs and glycemia control, our results indicate that measurement of insulin secretion is a valid end point for studies of this disease. Calculation of insulin secretion in response to a MMTT identified two patterns of response among individuals with type 1 diabetes, based on the timing of peak insulin secretion to the mixed meal. The pattern of response was not random but instead was consistently seen in the majority of individuals studied for 2 years. Moreover, the pattern of response was predictive of the progression of disease. Thus, studies of insulin secretory response in patients with type 1 diabetes may provide insights into disease mechanisms and have important implications for the design of clinical trials.

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