Impaired β-Cell Function, Incretin Effect, and Glucagon Suppression in Patients With Type 1 Diabetes Who Have Normal Fasting Glucose

Carla J. Greenbaum,1 Ronald L. Prigeon,2 and David A. D’Alessio3

We have recently described a novel phenotype in a group of subjects with type 1 diabetes that is manifested by glucose >11.1 mmol/l 120 min after an oral glucose load, but with normal fasting glucose levels. We now describe the metabolic characteristics of these subjects by comparing parameters of islet hormone secretion and glucose disposal in these subjects to age-matched nondiabetic control subjects. The patients with type 1 diabetes had fasting glucose, insulin, and glucagon values similar to those of control subjects. Additionally, the insulin secretory response to intravenous arginine at euglycemia was similar in the control and diabetic groups (264 ± 33.5 and 193 ± 61.3 pmol/l; P = 0.3). However, marked differences in β-cell function were found in response to hyperglycemia. Specifically, the first-phase insulin response was lower in diabetic subjects (329.1 ± 39.6 vs. 91.3 ± 34.1 pmol/l; P < 0.001), as was the slope of glucose potentiation of the insulin response to arginine (102 ± 18.7 vs. 30.2 ± 6.1 pmol/l per mmol/l; P = 0.005) and the maximum insulin response to arginine (2,524 ± 413 vs. 629 ± 159 pmol/l; P = 0.001). Although plasma levels of glucagon-like peptide (GLP)-1 and gastric inhibitory peptide (GIP) did not differ between control and diabetic subjects, the incretin effect was lower in the diabetic patients (70.3 ± 5.4 vs. 52.1 ± 5.9%; P = 0.03). Finally, there was a lack of suppression of glucagon in the patients after both oral and intravenous glucose administration, which may have contributed to their postprandial hyperglycemia. Glucose effectiveness did not differ between patients and control subjects, nor did insulin sensitivity, although there was a tendency for the patients to be insulin resistant (9.18 ± 1.59 vs. 5.22 ± 1.17 pmol·1−1·min−1; P = 0.08). These data characterize a novel group of subjects with type 1 diabetes manifested solely by hyperglycemia following an oral glucose load in whom islet function is normal at euglycemia, but who have marked defects in both α- and β-cell secretion at hyperglycemia. This pattern of abnormalities may be characteristic of islet dysfunction early in the development of type 1 diabetes. Diabetes 51:951–957, 2002

An unchecked autoimmune process is thought to result in the progressive loss of β-cell function and the development of type 1 diabetes. Clinically, the disease often presents abruptly with severe, symptomatic hyperglycemia with or without ketoacidosis. Despite the apparent acute onset, it is currently thought that the destruction of islet β-cells occurs over a course of months to years, with affected subjects having variable periods of subclinical disease (1–6). Such people can be identified before the onset of clinically apparent diabetes by the presence of islet autoantibodies and reduction of first-phase insulin secretion. We have recently described a group of subjects with type 1 diabetes who have a distinct profile of abnormal glucose regulation. In these individuals, fasting glucose levels are normal (<6.1 mmol/l), but severe hyperglycemia occurs following an oral glucose load (glucose >11.1 mmol/l 120 min after glucose ingestion) (7). These subjects are first- and second-degree relatives of individuals with type 1 diabetes that was discovered during screening for the Diabetes Prevention Trial, type 1 (DPT-1). They have antibodies to islet cell antigens and were excluded from the DPT-1 because of their oral glucose tolerance test (OGTT) results.

Individuals with the unique metabolic profile of normal fasting glucose but diabetic oral glucose tolerance comprised only a minority of the subjects evaluated for the DPT-1 (~10%). However, we have noted that many of these individuals retain this distinct metabolic profile over periods of months to years (8). Thus, these individuals are at an intermediate stage between a preclinical high-risk state and typical type 1 diabetes with elevated fasting and postprandial glucose levels. As such, they represent an opportunity for physiologic characterization of a novel stage of autoimmune diabetes. Therefore, we sought to determine the processes regulating glucose metabolism that permit these subjects to maintain fasting euglycemia while being unable to regulate glycemia following a glucose challenge. Specifically, we assessed β-cell secretion in response to glucose and arginine, the regulation of glucagon release, the incretin effect (the insulinotropic actions of gastrointestinal hormones and neural stimuli to augment the β-cell response beyond glycemic stimulation.
had a mean age of 23.5 years. Differences from typical type 1 diabetic patients in whom both fasting and postload insulin values were obtained during the IVGTT as the mean of the incremental insulin concentrations above basal levels obtained during the first 10 min. The acute insulin response to glucose (AIRg) was computed from the insulin values during the IVGTT as two points (fasting glucose and glucose ~11–14 mmol/l). The insulin values used for this computation were the average of the two baseline samples just before the arginine injection. The second slope was a measure of glucose potentiation of the insulin response to arginine (13). This parameter was computed in the same manner as the insulin/glucose slope, except that AIRarg was used instead of the baseline insulin concentrations.

The incretin effect was calculated for each individual from the insulin responses during the OGTT and matched intravenous glucose infusion using the following formula (10):

\[ 100 \times (\text{insulin AUC}_{OGTT} - \text{insulin AUC}_{(\text{glucose})})/\text{insulin AUC}_{OGTT} \]

Statistical analysis. Comparisons of experimental determinations between two groups in which the data were distributed normally were made with two-tailed t tests, and the rank-sum test was used for data that did not follow a normal distribution. Comparisons of more than two groups were made using ANOVA with post hoc tests to determine differences between specific groups. Within-group comparisons of poststimulus to basal values were made using repeated-measures ANOVA. Results are expressed as means ± SE unless otherwise noted.

RESULTS

Oral glucose tolerance test. The control (n = 14) and type 1 diabetic (n = 17) subjects had normal fasting glucose values that did not differ: 5.1 ± 0.07 and 5.3 ± 0.15 mmol/l, respectively (P = 0.17). In contrast, glucose values following glucose ingestion were significantly elevated in type 1 diabetic subjects relative to control subjects (Fig. 1A). The control group had a peak glucose level of 8.19 ± 0.59 mmol/l at 60 min, and plasma levels had returned to near basal by the end of the sampling period. The type 1 diabetic group had glucose concentrations that plateaued at 13.32 ± 0.36 mmol/l at 60 min, and there was no tendency for normalization of glycemia even at 120 min in the diabetic group. The glucose AUC during the OGTT was more than twice as large in the type 1 diabetic subjects relative to control subjects (265 ± 39.0 and 779 ± 39.4 mmol · 1−1 · min−1; P < 0.001).

Fasting insulin values were not different in the control and type 1 diabetic subjects (55.4 ± 5.1 and 82.6 ± 15.1 pmol/l, respectively; P = 0.13). Following glucose ingestion, insulin values were greater in the control subjects than in type 1 diabetic subjects at 30 and 60 min (P < 0.05), whereas values at 90 and 120 min were not different between the groups (Fig. 1B). Over the course of the OGTT, the insulin AUC was significantly greater in control subjects relative to type 1 diabetic subjects (31,134 ± 3,114 vs. 17,116 ± 2,592 pmol · 1−1 · min−1; P = 0.001). Similar results were observed in C-peptide AUC values (118 ± 15 vs. 61 ± 13 pmol · 1−1 · min−1; P = 0.007).

Fasting glucagon levels were similar in the control (n = 14), type 1 diabetic (n = 17) subjects, and the group was included for comparisons of OGTT results.
and type 1 diabetic (n = 8) groups (50.1 ± 3.7 and 45.8 ± 5.4 ng/l, respectively; P = 0.54). Following glucose ingestion, there was a significant decrease in plasma glucagon at 30 and 60 min in the control group, but no change relative to fasting levels in the type 1 diabetic group. Consistent with a lack of suppression of glucagon in the type 1 diabetic subjects, the concentrations of glucagon relative to fasting levels were different between the two groups at 60 min (control subjects, 68 ± 6% of basal; type 1 diabetic subjects, 92 ± 4% of basal; P = 0.05) (Fig. 1C).

There were no differences in fasting or postprandial levels of GIP and GLP-1 between the control (n = 10) and type 1 diabetic (n = 8) groups. Basal levels of GIP were 23.4 ± 2.7 and 21.6 ± 2.3 pmol/l in the control and type 1 diabetic subjects, respectively, and the responses (AUC) following glucose ingestion were not different between the groups (1,874 ± 358 and 1,378 ± 165 pmol · l⁻¹ · min⁻¹; P = 0.74). Fasting concentrations of GLP-1 were 9.3 ± 1.4 and 6.62 ± 0.76 pmol/l in the control and type 1 diabetic subjects, respectively (P = 0.15) and increased postprandially to a similar extent, with AUCs of 485 ± 70 and 677 ± 103 pmol · l⁻¹ · min⁻¹ (P = 0.12).

**Incretin effect.** Glucose values were well matched between the IVGTT and OGTT and equaled 103.1 ± 2 and 102.6 ± 2% in the control (n = 10) and type 1 diabetic (n = 8) groups, respectively. In both the control and type 1 diabetic subjects, insulin release was significantly greater during oral glucose ingestion than during intravenous glucose administration. However, the incretin effect constituted a significantly higher percentage of postprandial insulin release in the control group, 70.3 ± 5.4%, compared with the type 1 diabetic group, 52.1 ± 5.9% (P = 0.03). The impairment of the incretin effect in the type 1 diabetic group was most pronounced at 60 min after glucose ingestion, when it was about half of the response in control subjects (control subjects, 57.7 ± 6% type 1 diabetic subjects, 31.2 ± 6%; P = 0.01) (Fig. 2).

**IVGTT.** The acute insulin response to a glucose bolus (AIR₇) was diminished to a similar extent in both the type 1 diabetic (n = 8) and DPT (n = 8) subjects compared with control subjects (n = 10) (Fig. 3A), and this may have contributed to decreased rates of glucose disappearance (K_gl) in these individuals (Table 1). S_I and S_G derived from the IVGTT data were not different among the three groups (Table 1). Fasting glucagon levels before the IVGTT were similar in the control, DPT, and type 1 diabetic subjects.
(70.7 ± 5.7, 74 ± 2.0, and 68 ± 4.9 ng/l, respectively). Following intravenous glucose administration, glucagon levels were suppressed in the control and DPT groups (decremental AUC: −123.5 ± 18.7 and −72.3 ± 18 ng·l⁻¹·min⁻¹, respectively), but not in the type 1 diabetic subjects (19.9 ± 18.1 ng·l⁻¹·min⁻¹; P < 0.05 compared with control subjects) (Fig. 3B).

**Insulin/glucose slope and glucose potentiation.** Basal levels of glucose and insulin did not differ between the type 1 diabetic (n = 8) and control (n = 10) subjects, and AIR₇₀ at fasting glycaemia was also similar in the two groups (Table 2). In contrast, the type 1 diabetic subjects showed much lower insulin secretion at each level of hyperglycaemia. Thus, the insulin/glucose slope was significantly decreased in type 1 diabetic subjects (6.77 ± 1.72 pmol/l per mmol/l) relative to control subjects (30.2 ± 7.3 pmol/l per mmol/l; P = 0.013). In addition, the slope of glucose potentiation was also lower in the type 1 diabetic group (Table 2; Fig. 4). Finally, the AIR₇₀ at >22 mmol/l glucose (AIR₇₀) for the type 1 diabetic group was only 25% that of the control group (Table 2; Fig. 4).

**DISCUSSION**

This study reports a detailed investigation into the regulation of blood glucose in a unique group of subjects whose diabetes is best categorized as type 1 but is manifested solely by postprandial hyperglycaemia (7). These subjects were identified through the screening and staging process for DPT-1 and thus have evidence of islet autoimmunity and an impairment of first-phase insulin secretion in response to glucose. However, in contrast to DPT-1 subjects who have nondiabetic glucose tolerance, the subjects we have characterized in these studies have marked hyperglycaemia following an oral glucose load while having normal fasting glucose levels. Thus, the type 1 diabetic subjects represent a stage intermediate between the compensated glucose metabolism of the DPT-1 subjects and the totally uncompensated glucose metabolism of typical patients with type 1 diabetes. Both the DPT-1 subjects and the type 1 diabetic group we have described in this article are at high risk to progress to more severe, insulinopenic type 1 diabetes (7,18). This diabetes phenotype is novel and demonstrates that during the progression of autoimmune diabetes, normal fasting glucose can be maintained even in the face of a decrease in β-cell mass and a range of functional islet secretory defects.

We have demonstrated several parameters that potentially explain the disparity between fasting and postprandial glucose regulation in our type 1 diabetic group. As might be expected in the context of the serologic markers of β-cell autoimmunity, the type 1 diabetic subjects have evidence suggestive of a decrease in β-cell mass. The AIR₇₀ at glucose levels >22 mmol/l (AIR₇₀) has been shown in previous studies to correlate well with more direct assessments of β-cell number (13,19). The decrease of AIR₇₀ in the type 1 diabetic subjects is consistent with the loss of a significant fraction of β-cells, likely from autoimmune destruction. In addition, the diabetic subjects described herein have normal function when measured at euglycemia but a marked decrease in function when measured at hyperglycaemia. We have identified three other abnormalities that contribute to the abnormal OGTT. First, the β-cells in the type 1 diabetic subjects have a generally impaired responsiveness to glucose. Second, although the level of the incretin hormones GLP-1 and GIP are normal, the degree of augmented insulin secretion during oral glucose ingestion is below normal. Third, there is an abnormality in the suppression of glucagon during hyperglycaemia in the type 1 diabetic group, which would tend to promote hyperglycaemia.

As expected from the DPT-1 screening, the type 1 diabetic subjects had a markedly diminished FPIR as measured by AIR₇₀ relative to control subjects. Furthermore, the insulin/glucose slope and the slope of potentiation, measures of the effectiveness of glucose to augment insulin secretion in the presence of other secretagogues

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Type 1 diabetic subjects</th>
<th>DPT-1 subjects</th>
<th>Control subjects</th>
<th>Type 1 diabetic vs. DPT-1 subjects (P)</th>
<th>Type 1 diabetic vs. control subjects (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIR₀ (pmol/l)</td>
<td>91.3 ± 34.1</td>
<td>160.3 ± 50.3</td>
<td>329.1 ± 39.6</td>
<td>0.28</td>
<td>0.0005</td>
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<tr>
<td>K₆ (%/min)</td>
<td>1.07 ± 0.19</td>
<td>1.44 ± 0.21</td>
<td>2.74 ± 0.52</td>
<td>0.22</td>
<td>0.02</td>
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<tr>
<td>S₆ (min⁻¹)</td>
<td>0.017 ± 0.002</td>
<td>0.015 ± 0.0028</td>
<td>0.022</td>
<td>0.68</td>
<td>0.30</td>
</tr>
<tr>
<td>S₆ [×10⁻⁵·(pmol/l)⁻¹·min⁻¹]</td>
<td>5.22 ± 1.17</td>
<td>6.78 ± 0.956</td>
<td>9.18 ± 1.59</td>
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</tr>
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</table>

Data are means ± SE.

**TABLE 2**

<table>
<thead>
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<th></th>
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<th>Control subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Fasting AIR₇₀ (pmol/l)</td>
<td>193 ± 61.3</td>
<td>264 ± 33.5</td>
<td>0.30</td>
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<tr>
<td>AIR₇₀ (pmol/l)</td>
<td>629 ± 159</td>
<td>2524 ± 413</td>
<td>0.0013</td>
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<tr>
<td>Slope insulin/glucose (pmol/l per mmol/l)</td>
<td>6.77 ± 1.72</td>
<td>30.2 ± 7.3</td>
<td>0.013</td>
</tr>
<tr>
<td>Slope glucose potentiation (pmol/l per mmol/l)</td>
<td>30.2 ± 6.1</td>
<td>102 ± 18.7</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Data are means ± SE.
(13), were also significantly impaired in these individuals. However, since the acute insulin response to arginine at basal glucose was not different in these subjects relative to control subjects, it appears that their response to some nonglucose stimuli remains intact. The disproportionate defect in the insulin response to glucose in the type 1 diabetic subjects bears some similarity to the secretory abnormalities characteristic of type 2 diabetes (20), a condition in which β-cell dysfunction rather than β-cell loss is thought to be the primary pathology. This suggests that beyond islet cell destruction, the autoimmune process active in the type 1 diabetic subjects leads to functional compromise of the remaining β-cells. The presence of functional alterations in insulin secretion, in addition to β-cell loss, has been previously proposed in animal models (21) and in a small number of subjects with typical type 1 diabetes (22).

Insulin secretion following glucose ingestion was attenuated in the first hour of the OGTT in the type 1 diabetic subjects and increased only as plasma glucose levels became abnormally elevated. In addition, the type 1 diabetic group had a significant decrease in the incretin effect that was also most prominent in the first part of the OGTT. The incretin effect, the insulinotropic actions of gastrointestinal hormones and neural stimuli to augment the secretory function caused by the autoimmune process may be present in type 1 diabetes. Alternatively, the insulin-mediated suppression of α-cell secretion may be abnormal either because of lower intra-islet insulin levels or because the autoimmune milieu attenuates the paracrine effects of the secreted insulin. Although elevations in plasma glucagon do not have a measurable effect on postprandial glycemia in healthy humans (26), the contribution of abnormal glucagon suppression to glucose intolerance in diabetic subjects has been reported (27–29). We do not think the persistence of basal glucagon levels during the OGTT is likely to be the primary factor in the postprandial glycemic pattern in our type 1 diabetic subjects. However, as previously suggested (27–29), it is reasonable to believe that the relative hyperglucagonemia during the OGTT in the type 1 diabetic group contributes to their hyperglycemia, probably through persistent stimulation of hepatic glucose production.

Because of restrictions in access to subjects enrolled in the DPT-1, we were not able to perform incretin or glucose potentiation studies on this group. However, a more thorough examination of DPT-1 subjects would be very informative in discerning the relationship of the abnormalities we have described in type 1 diabetic subjects and oral glucose tolerance. One could speculate that AIRmax values in the DPT-1 group would fall between those of normal subjects and the type 1 diabetic group, suggesting that those antibody-positive subjects with normal glucose tolerance may simply have lost fewer islet cells than others. Alternatively, it may be that there are functional differences in β-cell secretion that contribute to postprandial hyperglycemia in groups of subjects with similar levels of islet autoimmunity. Based on the marked difference in OGTT between type 1 diabetic and DPT-1 subjects, and the defective incretin effect with type 1 diabetic subjects, we hypothesize that the incretin response in the latter group would have been closer to normal. This and other hypotheses will be tested in future studies, but our current findings indicate that there is heterogeneity in islet cell function among individuals with previous or ongoing islet autoimmunity.

The metabolic responses described in the type 1 dia-
Diabetic population are similar to subjects with type 1 diabetes who have a transient remission (or honeymoon) period (30–33). Diabetic patients who have a honeymoon phase can maintain normal HbA1c without insulin therapy for periods of months to years. Similar to our subjects, they continue to have very low or absent C-peptide responses to intravenous or oral glucose, yet they have normal responses to intravenous arginine and glucagon stimulation (30–33). To our knowledge, the incretin effect has not been measured in type 1 diabetic subjects during the honeymoon phase of their disease. A common feature of both our type 1 diabetic patients and those in the honeymoon phase is the absence of fasting hyperglycemia, which may facilitate effective β-cell function in the face of diminished β-cell mass. Alternatively, it is possible that the observed β-cell responses in both our type 1 diabetic patients and those in the honeymoon phase reflect a quiescence in the inflammatory activity surrounding the islet.

Interestingly, the OGTT responses of the type 1 diabetic subjects share some general features of classic impaired glucose tolerance and type 2 diabetes as well as other forms of nonautoimmune diabetes. The islet cell abnormalities in the type 1 diabetic group are similar in many respects to the characteristics of islet function in persons with impaired glucose tolerance (34,35) and type 2 diabetes. That is, a disproportionate loss of β-cell sensitivity to glucose, an impaired incretin effect, and dysregulation of glucagon release have all been previously described in type 2 diabetes (19,25) and are thought to contribute to glucose intolerance in those patients. However, there are important differences in our type 1 diabetic subjects and people with type 2 diabetes. The discrepancy between fasting glucose levels and postprandial glucose levels is much higher in type 1 diabetic than in type 2 diabetic subjects, where fasting glucose levels tend to increase in parallel with worsening glucose tolerance (36,37). In addition, whereas insulin resistance is a hallmark of type 2 diabetes (38), and several reports suggest that glucose effectiveness is impaired in these patients as well (39,40), these parameters did not differ among type 1 diabetic, control, or DPT-1 subjects in the present study. These type 1 diabetic subjects are also clearly distinct from individuals with maturity-onset diabetes of the young (MODY), another group of young subjects with mild diabetes stemming primarily from β-cell dysfunction. Individuals with mutations that classify them as having MODY1 and -2 typically have fasting hyperglycemia in addition to abnormal glucose tolerance, and MODY2 subjects have a decrease in β-cell sensitivity to glucose as a result of mutations in the glucokinase gene (41). Thus, clear differences in metabolic physiology can be demonstrated among type 1 diabetic subjects and other groups that share general phenotypic characteristics. Such heterogeneity emphasizes the value of in vivo characterization in the pursuit of the pathogenesis of different diabetic syndromes.

In summary, we have characterized important parameters of glucose tolerance in subjects with type 1 diabetes in whom hyperglycemia in response to oral glucose is the sole abnormality. These subjects demonstrate apparently normal islet function in the fasting state but show marked abnormalities in response to a glucose challenge. Not only is the β-cell response to hyperglycemia impaired, there is also a decreased incretin effect and a defect in the suppression of glucagon levels. It is possible that the ability of these subjects to maintain normal fasting glucose in the face of marked β-cell secretory abnormalities is at least in part due to their normal insulin sensitivity. These data demonstrate a novel profile of abnormalities in glucose regulation in a group of subjects that we propose have an intermediate, or early, stage of type 1 diabetes. These findings emphasize the gradations that are present in the phenotypic expression of type 1 diabetes and identify some of the more proximal functional abnormalities that occur with islet autoimmunity. The heterogeneity in islet cell function among subjects classified as having type 1 diabetes will need to be carefully considered in the design of future intervention trials.

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