Effect of Insulin-Glucose Infusions on Plasma Glucagon Levels in Fasting Diabetics and Nondiabetics

PHILIP RASKIN, YOSHIKUNI FUJITA, AND ROGER H. UNGER

From the Veterans Administration Hospital and the Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas 75235

ABSTRACT The effect of the intravenous infusion of insulin plus glucose on plasma glucagon levels was studied in hyperglycemic fasting adult-type and juvenile-type diabetics and compared with fasting nondiabetics. Adult-type diabetics were given insulin for 2 h at a rate of 0.03 U/kg·min, raising their mean insulin to between 25 and 35 μU/ml; glucagon declined from a base-line value of 71±2 (SEM) to 56±1 pg/ml at 120 min (P < 0.001). In juvenile-type diabetics given the same insulin-glucose infusion, glucagon declined from a base-line level of 74±8 to 55±5 pg/ml at 120 min (P < 0.05). The absolute glucagon values in the diabetic groups did not differ significantly at any point from the mean glucagon levels in nondiabetics given insulin at the same rate plus enough glucose to maintain normoglycemia. When glucagon was expressed as percent of baseline, however, the normoglycemic nondiabetics exhibited significantly lower values than adult-type diabetics at 90 and 120 min and juvenile-type diabetics at 60 min. In nondiabetics given insulin plus glucose at a rate that caused hyperglycemia averaging between 134 and 160 mg/dl, glucagon fell to 41±7 pg/ml at 120 min, significantly below the adult diabetics at 90 and 120 min (P < 0.01 and <0.05) and the juvenile group at 60 min (P < 0.01). The mean minimal level of 39±2 pg/ml was significantly below the adult (P < 0.001) and juvenile groups (P < 0.05). When insulin was infused in the diabetic groups at a rate of 0.4 U/kg·min together with glucose, raising mean plasma insulin to between 300 and 600 μU/ml, differences from the hyperglycemic nondiabetics were no longer statistically significant.

It is concluded that, contrary to the previously reported lack of insulin effect in diabetics during carbohydrate meals, intravenous administration for 2 h of physiologic amounts of insulin plus glucose is accompanied in unfed diabetics by a substantial decline in plasma glucagon. These levels are significantly above hyperglycemic nondiabetics at certain points but differ from normoglycemic nondiabetics only when expressed as percent of the baseline. At a supraphysiologic rate of insulin infusion in diabetics, these differences disappear.

INTRODUCTION

Hyperglucagonemia relative to the prevailing plasma glucose level has been reported in all forms of diabetes thus far examined. This includes spontaneous human diabetes of both juvenile-onset and adult-onset types (1-4) and insulin deficiency produced experimentally by alloxan (5, 6), streptozotocin (7), mannheptulose (5), anti-insulin serum (5), and diazoxide (5, 8). In alloxan diabetes the hyperglucagonemia is promptly corrected by modest quantities of insulin (5) even when the diabetes is of long duration (9). However, in human diabetes of the adult-onset type it was reported that the hyperglucagonemia is not corrected by exogenous insulin infused in supraphysiologic amounts during a large carbohydrate meal and decreases only sluggishly when pharmacologic doses are administered intravenously together with glucose in unfed patients (10). It was, therefore, suggested that the α-cell abnormality in spontaneous human diabetes, unlike that of alloxan diabetes, was not the simple consequence of insulin deficiency but rather represented a more complex disorder, perhaps even a primary inability of α-cells to respond to
insulin (10). The potential importance of this issue prompted a reexamination of the effect of insulin upon the α-cell response of human diabetics.

**METHODS**

27 diabetic patients were assembled from the Parkland Memorial Hospital Diabetes Clinic, Dallas, Tex. 12 were classified as ketoacidosis-prone, juvenile-onset type diabetics. Five were males, and seven were females. Ages ranged from 15 to 53 yr and averaged 33. Weights ranged from 46 to 90 kg and averaged 66 kg. Nine were non-obese, while two exceeded by 22% and 35% the normal weight standards of the Metropolitan Life Insurance Company. All required neutral protamine Hagedorn insulin, and the doses ranged from 20 to 95 U/day. There was no clinical evidence pointing to pancreatitis as the cause of the disease in any patient.

15 patients were classified as ketoacidosis-resistant, adult-onset type diabetics. 2 were male, and 13 were female. Their ages ranged from 39 to 64 yr and averaged 51 yr. Their body weights averaged 66 kg and ranged from 53 to 81 kg. Three were obese, exceeding the normal standard by 26, 11, and 20%. Seven others were slightly obese, less than 10% above the normal. 14 were receiving tolbutamide alone or in combination with phenformin at the time of the study; one was being treated with insulin.

13 nondiabetic subjects were assembled from among medical students and laboratory technicians; all were males. Their ages ranged from 27 to 38 yr, with an average age of 33. Body weights averaged 71 kg, and none were obese. Family history of diabetes mellitus was negative in all.

All subjects were studied as outpatients after an overnight fast. The diabetic subjects omitted all medications, including insulin and oral antidiabetic agents, on the morning of their test. All experiments were carried out with the informed consent of the subject.

A 20-min rest period preceded the start of all experiments. Normal subjects received 0.03 U/kg of glucagon-free crystalline insulin1 per hour together with a 5 to 15% glucose solution given at a rate intended either to maintain normoglycemia or to produce hyperglycemia and varying in the different experiments from 0.25 to 1.7 g/ kg·h. In the diabetic groups insulin was infused for 2 or 3 h at a rate of either 0.4 U/kg·h or 0.03 U/kg·h. Glucose was given at a rate intended to prevent a change in plasma glucose concentration and ranged from 0.17 to 0.6 g/kg·h.

Blood samples were obtained through an 18-gauge butterfly needle in the antecubital vein, and specimens were collected in chilled tubes containing 12 mg EDTA and 1 ml of Trasylol (500 kallikrein inhibitor U/ml of blood) and centrifuged promptly at 4°C. Plasma was separated and stored at −20°C until the time of hormone assay.

Glucagon was assayed by a recent modification (11) of the previously described radioimmunoassay (12) using antiserum G-58, which is highly specific for pancreatic glucagon and can distinguish differences of 10 pg/ml with 95% confidence. Insulin was measured by the Herbert modification (13) of the method of Yalow and Berson (14) in the nondiabetic subjects and in 12 of the 15 adult-onset type diabetics who did not have insulin antibodies in the plasma. Insulin was not measured in any of the juvenile-type diabetics since all had antibodies to insulin. Glucose was measured by means of the glucose oxidase method with the Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.).

Statistical analysis was made by means of the Student t test for paired groups for comparison of differences within groups. The base-line values represented the mean of the three preinfusion samples. The t test for two groups was employed for comparisons between groups.

**RESULTS**

**Effect of exogenous insulin upon plasma glucagon in hyperglycemic adult-onset type diabetics.** To reexamine the previously reported response of glucagon to a high dose of insulin in hyperglycemic adult-onset type diabetics (10), 0.4 U/kg·h of insulin was infused in seven such patients together with 0.6 gm/kg·h of glucose, a rate intended to maintain the base-line plasma glucose concentration (Fig. 1A); however, mean glucose rose more than 50 mg/dl above the base-line value of approximately 210 mg/dl. Insulin ranged between 300 and 600 μU/ml throughout the 180 min. Plasma glucagon declined from the base-line level of 86±7 (SEM) to 72±6 pg/ml at 30 min (P<0.05) and 67±8 pg/ml at 60 min (P<0.01) and reached a nadir of 53±6 pg/ml at 90 min (P<0.01), remaining in this range until the end of the insulin infusion. In the earlier report of similar experiments in 10 such patients, glucagon had declined from approximately 97±11 to 75+10 pg/ml 90 min later (10).

To determine the effect of a more physiologic change in insulin concentration, 0.03 U/kg·h was infused in 10 adult-type diabetic patients for 2 h with 0.2 g of glucose/kg·h designed to maintain the base-line plasma glucose concentration (Fig. 1B); however, mean glucose rose about 30 mg/dl above the 245 mg/dl base-line value. Plasma insulin averaged between 25 and 35 μU/ml throughout the infusion. Plasma glucagon declined significantly from the base-line level of 71±2 to 65±3 pg/ml at 30 min (P<0.05) and reached a nadir of 56±1 pg/ml (P<0.001) at 120 min. After discontinuation of the insulin infusion, plasma glucagon gradually rose to 61±1 pg/ml at 180 min, still significantly below the control level (P<0.005).

**Effect of exogenous insulin upon plasma glucagon in hyperglycemic juvenile-onset type diabetics.** To determine the effect of insulin on plasma glucagon levels in juvenile-onset type diabetics, nine such patients were infused with 0.4 U/kg·h of glucagon-free insulin and 0.6 g/kg·h of glucose for 180 min. Mean plasma glucose concentration was maintained at the base-line level throughout the first 120 min. As shown in Fig. 2A, plasma glucagon declined significantly from the preinfusion value of 67±5 to 52±5 pg/ml (P<0.05) at 30 min and reached a nadir of 45±3 pg/ml (P<0.005) at 120 min, remaining at about this level thereafter. Clearly, this large dose of insulin resulted in prompt

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1 Kindly provided by Dr. R. J. Hostey of Eli Lilly and Company, Indianapolis, Ind.
Figure 1 Plasma glucagon, glucose, and insulin levels (mean±SEM) in adult-type diabetics during intravenous insulin-glucose infusions. (A) Insulin 0.4 U/kg·h; glucose 0.6 g/kg·h. (B) Insulin 0.03 U/kg·h; glucose 0.2 g/kg·h.

Figure 2 Plasma glucagon and glucose levels (mean±SEM) in juvenile-type diabetics during intravenous insulin-glucose infusions. (A) Insulin 0.4 U/kg·h; glucose 0.6-0.4 g/kg·h. (B) Insulin 0.03 U/kg·h; glucose 0.17 g/kg·h.

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and substantial lowering of glucagon in hyperglycemic juvenile-type diabetics.

To examine the effect of a physiological change in the concentration of exogenous insulin upon plasma glucagon levels in juvenile-onset type diabetics, 0.03 U/kg·h of glucagon-free insulin was infused in seven such patients over 120 min together with sufficient glucose to prevent a decline in hyperglycemia. As shown in Fig. 2B, plasma glucagon declined from the preinfusion level of 74±8 to 65±5 pg/ml at 60 min (P < 0.05), reaching a nadir of 55±5 pg/ml at 120 min (P < 0.05). After discontinuation of the infusion, plasma glucagon rose slightly to 65±6 pg/ml at 150 min but remained below the base-line value (P < 0.05).

Effect of exogenous insulin upon plasma glucagon in normoglycemic and hyperglycemic nondiabetic controls.

To determine the effect of insulin on plasma glucagon levels in nondiabetics, insulin was administered in 10 normal subjects at a rate of 0.03 U/kg·min, and glucose was infused at approximately 0.25 g/kg·h, a rate intended to maintain the base-line glucose concentration. As shown in Fig. 3A, mean glucose remained relatively constant, ranging between 74±3 and 84±5 mg/dl throughout the infusion. Mean insulin ranged between 33±2 and 43±6 μU/ml during this period. Glucagon declined rapidly from 75±5 pg/ml before the infusion to 55±4 pg/ml at 60 min (P < 0.001) and remained very close to its 120-min nadir of 50±3 pg/ml (P < 0.001) thereafter. 30 min after discontinuation of the insulin-glucose infusion, at which time plasma glucose had declined to 67 mg/dl and insulin to below 16 μU/ml, mean plasma glucagon rose from the nadir to 61±4 pg/ml at 180 min but still was significantly below the control level (P < 0.05).

To maintain sustained hyperglycemia, the rate of glucose infusion in five normal subjects was increased to 1.7 g/kg·h, while insulin was infused at the same rate of 0.03 U/kg·min. Mean plasma glucose levels rose to between 134±5 and 160±7 mg/dl (Fig. 3B), and mean insulin levels increased to between 24±8 and 45±4 μU/ml during this 2-h period. These insulin levels did not differ significantly from those measured in the adult-type diabetics given the same rate of glucose infusion. (Nor did they differ significantly from those of the normoglycemic nondiabetics, perhaps because of suppression by exogenous insulin of the endogenous insulin response to the relatively weak stimulus of intravenous glucose.) Glucagon declined from the base-line value.
TABLE I

Plasma Glucagon before and during Insulin-Glucose Infusions in Normoglycemic and Hyperglycemic Nondiabetics and Hyperglycemic Diabetics

<table>
<thead>
<tr>
<th></th>
<th>Mean baseline</th>
<th>Time (min)</th>
<th>Mean nadir</th>
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<tr>
<td></td>
<td></td>
<td>-30</td>
<td>-15</td>
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<tr>
<td>pg/ml</td>
<td></td>
<td></td>
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<tr>
<td>Nondiabetics</td>
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<td></td>
<td></td>
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<tr>
<td>Normoglycemic</td>
<td>75±5</td>
<td>76±6</td>
<td>77±7</td>
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<td>Hyperglycemic</td>
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<td>73±8</td>
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<tr>
<td>Adult-onset type diabetics</td>
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<tr>
<td>Low insulin</td>
<td>72±2</td>
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<td>High insulin</td>
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<td>Juvenile-onset type diabetics</td>
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<tr>
<td>Low insulin</td>
<td>77±10</td>
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<td>78±11</td>
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<tr>
<td>High insulin</td>
<td>69±5</td>
<td>72±6</td>
<td>69±7</td>
</tr>
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* P < 0.01 vs. hyperglycemic nondiabetics.
† P < 0.02 vs. hyperglycemic nondiabetics.
‡ P < 0.05 vs. hyperglycemic nondiabetics.
¶ P < 0.001 vs. hyperglycemic nondiabetics.

level of 73±8 pg/ml to a nadir of 41±7 pg/ml at 120 min (P < 0.05). This was not significantly lower than in the normoglycemic nondiabetics.

Comparison of glucagon responses to insulin in nondiabetics and diabetics. Table I records the mean plasma glucagon values at all time points before, during, and after the insulin-glucose infusions in all groups, expressed in picograms per milliliter. In addition the mean nadir is given. There were no significant differences between normoglycemic and hyperglycemic nondiabetics at any time point before or after the start of the infusions. In addition, there was no significant difference between the plasma glucagon values of the normoglycemic group of nondiabetics and any of the diabetic groups at any time point. Only when the hyperglycemic nondiabetics were compared with diabetics receiving the same dose of insulin were significant differences in absolute glucagon levels observed. In the hyperglycemic nondiabetics mean plasma glucagon had declined at 60 min to 46±4 pg/ml, significantly below both of the diabetic groups receiving insulin at the same rate (P < 0.01), and this difference was also evident in the adult group at 90 (P < 0.01) and 120 min (P < 0.05). Statistically significant differences between glucagon levels of the hyperglycemic nondiabetics and the juvenile-type diabetic group were present.

**FIGURE 4** Plasma glucagon levels as percent of base-line values (±SEM) during intravenous insulin-glucose infusion in (A) nondiabetics, (B) adult-type diabetics, and (C) juvenile-type diabetics. Asterisks indicate significant differences resulting from varying experimental conditions within a group (*P < 0.05; **P < 0.01); letters indicate differences between diabetics and normoglycemic nondiabetics (n, P < 0.05; N, P < 0.01) and hyperglycemic nondiabetics (h, P < 0.05; H, P < 0.01).

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only at 60 min \( (P < 0.01) \), although mean glucagon levels remained at 55 pg/ml or above thereafter, substantially above the nondiabetics. The average minimal glucagon level in the hyperglycemic nondiabetic group was 39±3 pg/ml, significantly below the 54±1-\( (P < 0.001) \) and 53±4-pg/ml \( (P < 0.05) \) average minimal level of the adult- and juvenile-type diabetics, respectively.

In Fig. 4, plasma glucagon levels were expressed as percent of the basal values. Differences between adult-type diabetics at the low insulin dose and hyperglycemic nondiabetics became more significant, and differences between normoglycemic diabetics appeared at 90 and 120 min. The juvenile-type diabetics at the low insulin dose now differed from hyperglycemic nondiabetics at 60 and 120 min and from the normoglycemic nondiabetics at 60 min.

In the adult-type diabetics, the higher rate of insulin infusion reduced the mean glucagon levels significantly below those of the lower-rate infusion at 90 \( (P < 0.05) \) and 120 min \( (P < 0.01) \) but only when expressed as percent of baseline (Fig. 4). At the higher dose the mean glucagon levels of these diabetics no longer differed significantly from the hyperglycemic normals at individual time points or at the nadir. In the juvenile-type diabetic group, the higher rate of insulin infusion was accompanied by a significant difference in glucagon from the lower infusion rate at 30 min \( (P < 0.05) \) only when expressed as percent of baseline (Fig. 4); the absolute values were as low as those of the normoglycemic normals.

Although the difference was not statistically significant mean plasma glucagon was suppressed to a greater extent in the hyperglycemic nondiabetics than in the normoglycemic group (Fig. 4).

**DISCUSSION**

While confirming earlier evidence of diminished suppressibility of plasma glucagon in human diabetics \( (10) \), the present study forces modifications in the previous interpretation that low levels of exogenous insulin do not reduce plasma glucagon in such patients. That assumption was based on the fact that a constant intravenous infusion of insulin, which raised mean plasma insulin levels to approximately 300 \( \mu \)U/ml, failed to reduce plasma glucagon in a group of 10 adult diabetics during a large carbohydrate meal \( (10) \).

However, the present study indicates that glucagon is suppressible in both adult-onset type and juvenile-onset type diabetic patients in the unfed state when insulin levels are raised to between 24 and 45 \( \mu \)U/ml. However, a subtle insulin-independent defect in \( \alpha \)-cell function could still exist inasmuch as normalization of glucagon was not complete. Mean values of glucagon differed significantly from normoglycemic nondiabetic controls given the same dose of insulin plus enough glucose to prevent a fall in plasma glucose from the fasting state level only when expressed as percent of the baseline, and then at only two of four time points in the adult-type group and one of four in the juvenile-type group. When compared with values for nondiabetics made hyperglycemic the absolute mean glucagon values of diabetics differ significantly at various points and at the nadir; this difference was most striking in the adult-type diabetics and was enhanced when expressed as percent of baseline.

It can, of course, be argued that the induction of hyperglycemia in nondiabetics involves a sudden change from steady-state glycemia while the maintenance of hyperglycemia in the diabetics does not, making such comparisons invalid. Yet, the mean minimal level in hyperglycemic nondiabetics averaged 39±3 pg/ml, considerably below the mean minimal values reached in 2 h by the far more hyperglycemic diabetic groups given the same insulin dose. Only in the juvenile-type group given the supraphysiologic high insulin dose did glucagon decline to this range.

The reduction of plasma glucagon in hyperglycemic diabetics given a low-dose infusion of insulin to levels approximately those of normoglycemic nondiabetics given the same dose of insulin seemingly excludes complete \( \alpha \)-cell insensitivity to insulin. However, inability to reduce glucagon of diabetics to levels as low as in the hyperglycemic nondiabetics, at least in 2 h, could reflect an intrinsic difference in \( \alpha \)-cell function independent of plasma insulin levels, one that could be situated at any point between the receptors for glucose and/or insulin and the secretory process itself. However, the higher insulin levels of the nondiabetic subjects, although not significantly above the diabetics, could possibly account for the observed differences.

A much larger glucose load was required to maintain hyperglycemia in the nondiabetic group than in the diabetics. This could indicate greater general insulin sensitivity than in the diabetics, or it could signify more avid hepatic glucose uptake by the liver resulting from endogenous insulin secretion, most of which is bound by the liver on its initial circulation and, therefore, not reflected in the insulin measurements in the posthepatic plasma. The influence of the glucose load on these studies is not clear.

The fact that hyperglucagonemia of diabetes, to which many of the abnormalities of the disease have recently been attributed \( (15) \), was first observed in diabetic patients that were relatively well controlled by various of the currently employed methods of glucoregulation \( (1-3) \) first led to the assumption that relative hyperglucagonemia was independent of the insulin level. The
failure of glucagon to decline during the ingestion of a carbohydrate meal even when insulin levels were maintained well above normal (10) further supported this possibility. More recently, significant glucagon hyperresponsiveness to arginine has been reported in “prediabetics” with perfectly normal insulin levels and in hyperinsulinemic diabetics; this has been interpreted as evidence of an α-cell disorder that obviously cannot be ascribed to reduced insulin levels. In another study of “prediabetics,” however, although the glucagon response of arginine was also higher than in controls, the difference was not significant (16).

It is possible, however, that glucagon suppression below the basal secretory rate involves an insulin-dependent mechanism, while control of the aminogenic response involves a separate glucose-dependent, insulin-independent function that, in human diabetes, may be disturbed before measurable derangement in β-cell function is manifest.

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