# Diminished B Cell Secretory Capacity in Patients with Noninsulin-dependent Diabetes Mellitus

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**Abstract.** In order to assess whether patients with noninsulin-dependent diabetes mellitus (NIDDM) possess normal insulin secretory capacity, maximal B cell responsiveness to the potentiating effects of glucose was estimated in eight untreated patients with NIDDM and in eight nondiabetic controls. The acute insulin response to 5 g intravenous arginine was measured at five matched plasma glucose levels that ranged from ~100–615 mg/dl. The upper asymptote approached by acute insulin responses ($AIR_{max}$) and the plasma glucose concentration at half-maximal responsiveness ($PG_{50}$) were estimated using nonlinear regression to fit a modification of the Michaelis-Menten equation. In addition, glucagon responses to arginine were measured at these same glucose levels to compare maximal A cell suppression by hyperglycemia in diabetics and controls.

Insulin responses to arginine were lower in diabetics than in controls at all matched glucose levels ($P < 0.001$ at all levels). In addition, estimated $AIR_{max}$ was much lower in diabetics than in controls (83±21 vs. 450±93 μU/ml, $P < 0.01$). In contrast, $PG_{50}$ was similar in diabetics and controls (234±28 vs. 197±20 mg/dl, $P$ equals NS) and insulin responses in both groups approached or attained maxima at a glucose level of ~460 mg/dl. Acute glucagon responses to arginine in patients with NIDDM were significantly higher than responses in controls at all glucose levels. In addition, although glucagon responses in control subjects reached a minimum at a glucose level of ~460 mg/dl, responses in diabetics declined continuously throughout the glucose range and did not reach a minimum. Thus, A cell sensitivity to changes in glucose level may be diminished in patients with NIDDM.

In summary, patients with NIDDM possess markedly decreased maximal insulin responsiveness to the potentiating effects of glucose. Such a defect indicates the presence of a reduced B cell secretory capacity and suggests a marked generalized impairment of B cell function in patients with NIDDM.

## Introduction

Insulin secretion in patients with insulin-dependent diabetes mellitus is nearly or totally absent. In contrast, some measures of insulin secretion in patients with noninsulin-dependent diabetes mellitus (NIDDM),[1] such as the basal insulin level, the second-phase insulin response to glucose, and insulin responses to nonglucose stimuli, may appear to be similar to those of nondiabetic subjects (1). However, the magnitude of such measures is dependent upon the ambient glucose level. Thus, when compared to nondiabetic subjects at equal plasma glucose levels, patients with NIDDM possess consistently lower acute insulin responses to nonglucose secretagogues such as arginine or isoproterenol (2).

However, interpretation of these findings has been limited because the glucose level required for maximal insulin responses to nonglucose stimuli has not been previously determined in normal man or in patients with NIDDM. As a result, it is not clear whether the impairment of insulin secretory responses of patients with NIDDM can be overcome by a sufficiently high plasma glucose level. If patients with NIDDM have normal maximal responsiveness to glucose level, but a rightward

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1. *Abbreviations used in this paper:* $AIR_{max}$, maximal acute insulin response to arginine; $AGR_{min}$, minimum acute glucagon response to arginine; NIDDM, noninsulin-dependent diabetes mellitus; $PG_{50}$, plasma glucose concentration at half-maximal responsiveness.

shifted dose-response curve, it would suggest the presence of a glucosensory impairment that was capable of being overcome by very high glucose levels (3). Alternatively, if maximal responsiveness were decreased in NIDDM, it would suggest the presence of a defect in B cell secretory capacity due to diminished B cell number or generalized dysfunction of a normal number of B cells.

Increasing the plasma glucose level is known to decrease glucagon level and the glucagon secretory response to arginine (4, 5). As is the situation for insulin responses, it has not yet been determined how high the glucose level must be raised in order to completely suppress such responses in normal and in diabetic subjects.

Thus, in the present study, insulin and glucagon secretory responses to arginine were determined at five different glucose levels in order to define dose-response characteristics in patients with NIDDM and in nondiabetic controls.

## Methods

*Subjects.* Eight obese nondiabetic male volunteers and eight male patients with NIDDM gave written informed consent before participating in the present study. No control subject had medical illnesses, was taking medication, or had a history of diabetes mellitus in parents or siblings. As shown in Table I, control subjects and diabetic patients were of similar age (46±3 vs. 51±3 yr, mean±SEM) and percentage of ideal body weight (129±4 vs. 131±4, New York Metropolitan Life Insurance Tables, 1959). Fasting plasma glucose was 232±25 mg/dl in patients with NIDDM and 93±1 mg/dl in control subjects. Patients had a history of stable hyperglycemia for at least 3 yr, and all but one was treated by diet alone. One patient was chronically treated with chlorpropamide, which was discontinued 3 wk before the present study. No patients were on other medications except for a normokalemic patient who took hydrochlorothiazide, 50 mg/d, for essential hypertension. No patients or control subjects were alcoholic and none had a history of pancreatitis. Each subject consumed an *ad libitum* diet before each of his two studies and fasted, except for water, for 12 h before each study.

*Study protocol.* Studies were performed in the metabolic ward of the Seattle VA Medical Center. Blood samples were obtained via a 2-in, 16-gauge teflon catheter inserted into a superficial wrist vein. To achieve arterialization of venous blood, the hand and wrist from which the samples were obtained were warmed in a wooden box that was thermostatically heated to 60°C throughout the study (6). One-half of each glucose infusion was administered through a 12-in., 18.5 gauge teflon catheter inserted into an antecubital vein ipsilateral to the sampling vein. The other half of each glucose infusion was simultaneously given through a 2-in., 16-gauge teflon catheter which was placed in the contralateral forearm.

Each normal and each diabetic subject underwent two studies that were separated by at least 1 wk. The experimental protocol for normal subjects is depicted in Fig. 1. On the first study day for normal subjects, at 0900 h, two base-line blood samples were obtained 5 min apart for measurement of plasma insulin and glucose (4 ml), glucagon (3 ml), and norepinephrine and epinephrine (2.5 ml). Then, a maximally stimulating (7) dose if 10% arginine hydrochloride (5 g, Cutter Laboratories, Inc., Sacramento, CA) was administered as an intravenous injection over 30 s, the end of which was designated time zero. Blood samples were obtained 2, 3, 4, and 5 min after this and all other arginine doses for measurement of insulin and glucagon. After the 5-min sample was obtained, a variable rate infusion of 10% dextrose was administered via a peristaltic pump (Polystaltic, Haake-Buchler Instruments, Saddle Brook, NJ) in order to raise and maintain the plasma glucose level at ~230 mg/dl. Every 5 min, a blood sample was assayed for glucose level by use of a portable glucose analyzer (Beckman Instruments, Palo Alto, CA) and the infusion rate adjusted accordingly, as described previously (8). 30 min after beginning the glucose infusion, base-line blood samples were again obtained for measurement of insulin and glucagon, and acute insulin and glucagon responses to a 5-g arginine pulse were again measured. An intravenous arginine pulse does not inhibit subsequent insulin responses measured 30 min later in normal subjects (7) or in patients with NIDDM (9).

In order to avoid priming effects of hyperglycemia (10, 11), a 2-h glucose washout period followed the glucose infusion. 90 min after beginning the glucose washout period a blood glucose level was measured, and a variable rate glucose infusion (euglycemic clamp) was begun if the glucose level was equal to or lower than the fasting value. The purpose of this infusion was to clamp plasma glucose at the initial fasting level. 120 min after beginning the glucose washout period, blood samples were obtained for measurement of epinephrine, norepinephrine, insulin, glucagon, and glucose. Repeat insulin and glucagon responses to arginine were then measured at euglycemia in order to ascertain whether islet function had changed. The glucose infusion rate was then increased and adjusted to achieve and maintain a plasma glucose level of ~600 mg/dl. 30 min after beginning this high rate infusion, a fourth arginine pulse was injected and the insulin and glucagon responses measured. Catecholamine levels were again measured before administration of this arginine dose.

At least 1 wk later, each normal subject returned for a second study. The only difference was that glucose levels were clamped at ~345 and 460 mg/dl during the second and fourth pulses of arginine instead of at 230 and 615 mg/dl.

The experimental protocol for patients with NIDDM is depicted in Fig. 2. These patients also underwent measurement of insulin and glucagon responses to arginine at plasma glucose levels of ~120, 230, 345, 460, and 615 mg/dl. To achieve a glucose level of ~120 mg/dl, seven of the eight patients were given insulin infusions before measurement of the first insulin response to arginine. One patient had a fasting plasma glucose of 137 mg/dl on the first study day and therefore did not receive an insulin infusion).

On the first study day in diabetics, blood samples were obtained for insulin, glucagon, glucose, and catecholamines. An intravenous infusion of regular purified park insulin (Actrapid, Novo Laboratories, Wilton, CT) at a rate of 2.5 mU/kg per min was then initiated. Blood samples were subsequently obtained every 10 min for on-line measurement of blood glucose level, and when the glucose level fell to 120–140 mg/dl (40–80 min after beginning insulin), the insulin infusion was discontinued. A 60-min insulin washout period, during which glucose levels were monitored every 20 min, followed the insulin infusion. At the end of this period, blood samples were obtained for insulin, glucagon, glucose, and catecholamines. An acute insulin response to arginine was then measured at the target glucose level of ~120 mg/dl. The plasma glucose level was then raised to ~230 mg/dl for 30 min by a variable rate glucose infusion, and a second insulin response to arginine was measured at this level. Because a priming effect of hyperglycemia is minimal or absent in NIDDM (12), a glucose washout period did not follow this hyperglycemic clamp. Instead, the glucose

level was immediately raised and maintained at ~615 mg/dl for 30 min, where a third insulin response to arginine was measured.

At least 1 wk after this study, diabetic patients returned for a second study during which the first insulin response to arginine was measured at a plasma glucose level of ~345 mg/dl. In one patient, this response was measured at the fasting plasma glucose level. In the other seven, a 30-min variable rate glucose infusion was administered to achieve this glucose level. In all patients, a second insulin response to arginine was measured 30 min after clamping the glucose level at ~460 mg/dl. None of the diabetic or control subjects noticed any symptomatic discomfort during the administration of glucose or arginine.

Because insulin may inhibit its own secretion (13, 14), we performed on additional study in three of the patients with NIDDM in order to ascertain whether the insulin infusion would alter subsequently measured insulin and glucagon responses to arginine. In this study, after a baseline insulin and glucagon response to a 5-g arginine pulse was measured, an insulin infusion of 2.5 mU/kg per min was given for 60 min. During this insulin infusion and for the remainder of this control study, plasma glucose was clamped at the fasting level by a variable-rate glucose infusion. A 60-min insulin washout period followed the insulin infusion, after which a repeat insulin and glucagon response to arginine was obtained and compared with the initial insulin response. Insulin and glucagon responses were quite similar before and after the insulin infusion (mean insulin response to arginine: 80 vs. 85 $\mu$U/ml [mean±SEM of difference equals 7±3]; mean glucagon response to arginine: 154 vs. 149 pg/ml [mean±SEM of difference equals 9±6]; mean plasma glucose level: 269 vs. 266 mg/dl [mean±SEM of difference equals 4±1]; $P$ not significant for any of the above). Thus, it appears that given an adequate insulin washout period, an insulin infusion of 2.5 mU/kg per min in patients with NIDDM will not alter insulin or glucagon responses to arginine when plasma glucose levels are held constant.

*Analytical methods.* Plasma insulin levels were assayed by a modification of the double antibody method of Morgan and Lazarow (15). Plasma glucose was measured by an autoanalyzer using the glucose oxidase method (Technicon Instruments Corp., Tarrytown, NY). Plasma glucagon was measured by radioimmunoassay, employing a C-terminal-directed antiserum (16). Plasma epinephrine and norepinephrine levels were measured by single isotope enzymatic assay (17).

*Calculations and statistics.* Fasting plasma levels of glucose and insulin were calculated as the mean of the initial levels obtained on each of the two study days. Acute insulin and glucagon secretory responses to arginine were calculated as the mean of the hormone levels obtained 2–5 min after the arginine injection minus the mean of the prestimulus hormone levels. Hormone secretory responses obtained at the basal glucose level in normal subjects were calculated as the mean of the four responses obtained before and after the 2-h glucose washout periods on each of the two study days. The glucose potentiation slope was calculated as the difference between the insulin responses to arginine obtained at glucose levels of ~100 and 230 mg/dl divided by the difference between these two plasma glucose levels. Thus, the potentiation slope was measured over the early, most nearly linear portion of the plasma glucose vs. insulin response dose-response curve.

To determine whether insulin responses for each subject tended to approach a maximum as the glucose level was raised, the slopes of the final two dose-response curve segments were compared. If the slope of the segment connecting insulin responses at glucose levels of 460 and 615 mg/dl was less than the slope of the prior segment (from 345 to 460 mg/dl), the curve was deemed to be approaching a maximum. A similar method was used to ascertain if glucagon responses approached a minimum as plasma glucose increased. If the slope of the segment connecting glucagon responses at glucose levels of 460 and 615 mg/dl was less steep than the slope of the prior segment (from 345 to 460 mg/dl), the curve was designated as approaching a minimum.

In order to estimate maximal glucose potentiation of the insulin response to arginine, the maximal acute insulin response ($AIR_{max}$) and plasma glucose concentration at half-maximal responsiveness ($PG_{50}$) were estimated by use of the Michaelis-Menton equation as modified by Grodsky (18): $\widehat{AIR} = (AIR_{max} \cdot PG^K) \div (PG_{50}^K + PG^K)$. The exponent $K$ is directly related to the rate of rise of insulin response per unit change in glucose level. $K$ is the parameter which Grodsky incorporated into the Michaelis-Menten equation in order to accurately model insulin responses in rat islets. Parameter estimates were obtained by nonlinear least squares regression using the modified Gauss-Newton method (19). $\widehat{AIR}$ denotes the continuous estimate of acute insulin response obtained by such curve fitting. $AIR_{max}$ is the upper asymptote which such a curve approaches, and $PG_{50}$ is the glucose level at $(AIR_{max}) \div 2$.

For each subject, the curve fitting precision was estimated by $R^2$, the percentage reduction in the residual sum of squares when $\widehat{AIR}$ was used instead of the average observed response, $\overline{AIR}$, to predict the mean AIR at each glucose level. Thus, $R^2 = \{[\Sigma (AIR - \overline{AIR})^2 - \Sigma (AIR - \widehat{AIR})^2] \div [\Sigma (AIR - \overline{AIR})^2]\} \times 100$, and values of $R^2$ near 100 indicate a curve which comes extremely close to the observed data.

Dose-response parameters for glucagon responses to arginine as a function of increasing glucose levels in normal subjects were estimated using a modification of the above equation. This modification was employed so that a lower asymptote ($AGR_{min}$) representing maximal suppression of the glucagon response to arginine by hyperglycemia could be estimated: $AGR_{100} - \widehat{AGR} = [(AGR_{100} - AGR_{min}) \cdot PG^K] \div [PG_{50}^K + PG^K]$. For each subject, $AGR_{min}$, $PG_{50}$ (glucose level at the midpoint between the AGR at euglycemia [$AGR_{100}$] and $AGR_{min}$), and $K$ (an index of the rate of decline of AGR per unit increase in glucose level) were calculated. $\widehat{AGR}$ is the continuous estimate of acute glucagon response obtained by curve fitting. The glucagon response data was smoothed before curve fitting by the technique of 3-median smoothing as described by Tukey (20).

Except where designated otherwise, two-tailed $t$ test for independent samples was employed. A $P$ value of 0.05 was taken as the level of significance. Data are expressed as mean±SEM.

## Results

*Insulin secretion.* Mean insulin and glucose levels obtained before and after each arginine pulse are illustrated in Fig. 1 for control subjects and in Fig. 2 for diabetic patients. In controls, the initial insulin response to arginine obtained on the first study day, 57±13 $\mu$U/ml, was quite similar to the response determined after the 2-h washout period, 59±14 $\mu$U/ml (difference not significant by paired $t$ test). Similarly, the initial insulin response on the second study day in controls, 68±22 $\mu$U/ml, was not significantly different from the response determined after the glucose washout period, 74±11 $\mu$U/ml.

Prestimulus insulin levels and calculated insulin responses to arginine in normal and diabetic patients are depicted in Fig. 3*A* and *B*, respectively, as functions of plasma glucose level. It is clear that the mean insulin responses to arginine are much greater in the control group than in the diabetic group at all matched glucose levels ($P < 0.001$ at all levels). It also appears that mean insulin responses of both normal and

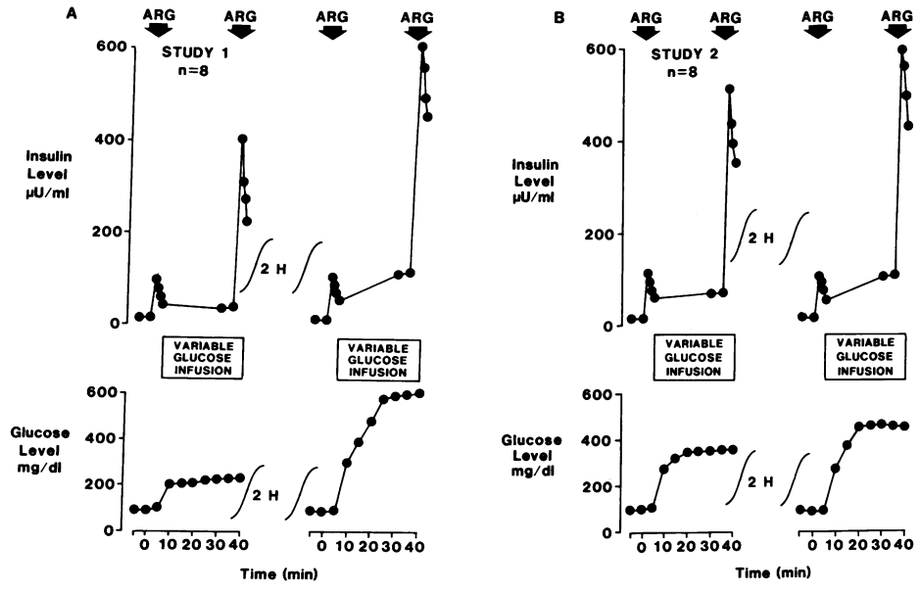


*Figure 1.* Experimental protocol in eight nondiabetic control subjects illustrating mean insulin and glucose levels. Pulses of intravenous arginine (ARG, 5 g) were administered after clamping plasma glucose at levels of ~95, 225, and 615 mg/dl (*A*) and at ~95, 355, and 462 mg/dl (*B*).

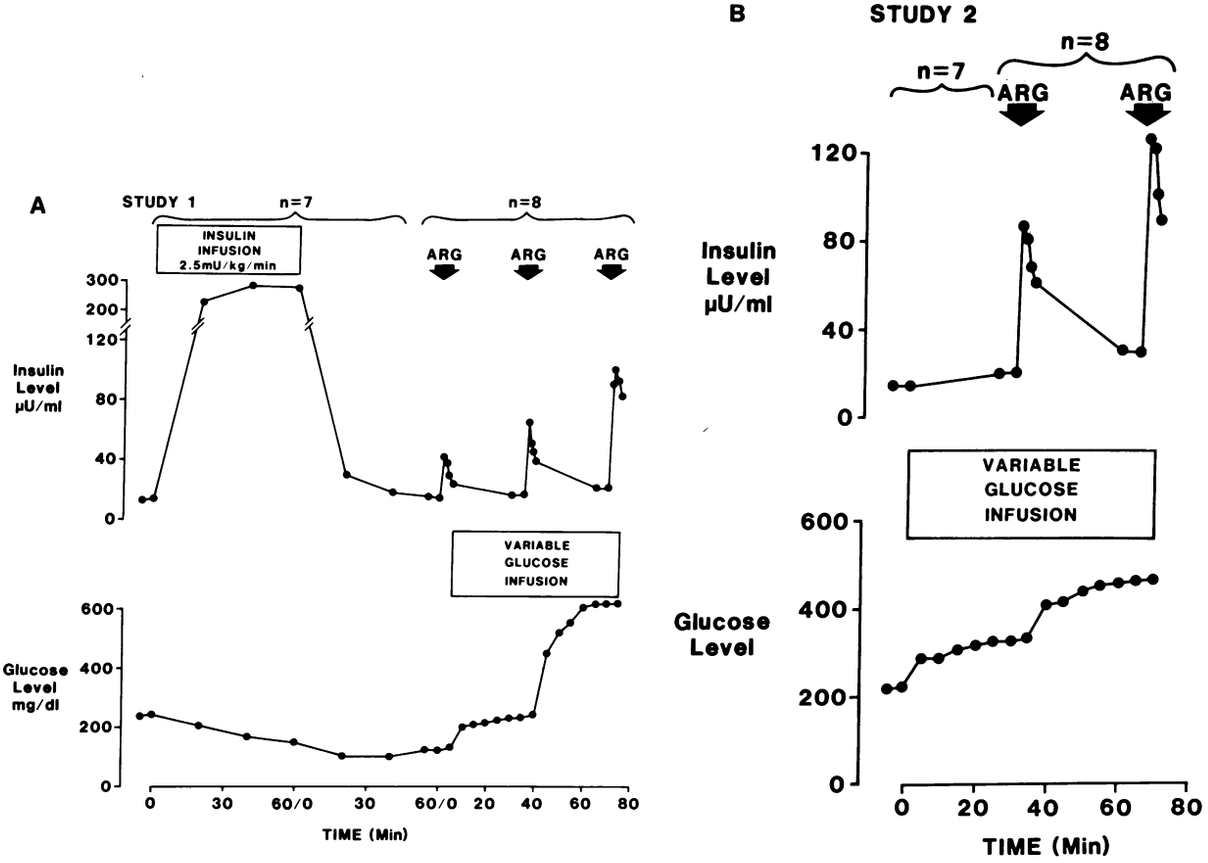


*Figure 2.* Experimental protocol in diabetic patients illustrating mean insulin and glucose levels. To achieve euglycemia, seven of eight patients required an insulin infusion, which was followed by a 60-min insulin washout period. Pulses of intravenous arginine (ARG, 5 g) were then administered at a glucose level of ~120 mg/dl and after clamping plasma glucose at 240 and 620 mg/dl (*A*). On a separate day, pulses of arginine were given after clamping plasma glucose at levels of ~330 and 460 mg/dl (*B*).

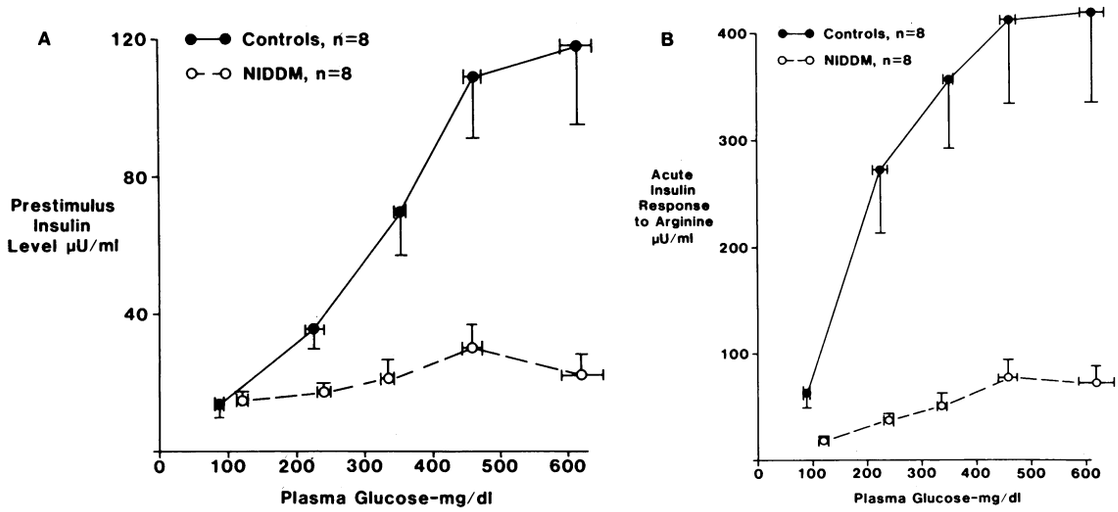


*Figure 3.* A comparison of prestimulus insulin levels (*A*) and acute insulin responses to arginine (*B*) at five plasma glucose levels in eight patients with NIDDM and in eight control subjects. The insulin responses to arginine were greater in the control group than in the diabetic group at all matched glucose levels ($P < 0.001$ at all levels). Data equals mean±SEM.

diabetic groups approach, or reach, a maximum at a glucose level of ~460 mg/dl. Elevation of plasma glucose level from ~460–615 mg/dl was accompanied by insignificant changes in mean insulin responses in the control group (from 411±79 to 419±86 μU/ml) and in the diabetic group (from 82±16 to 72±14 μU/ml). In order to ascertain if insulin responses of each individual tended to approach a maximum, slopes of the third and fourth segments of the glucose levels vs. insulin-

*Table I. A Comparison of Age, Percentage of Ideal Body Weight (IBW), Fasting Plasma Glucose (FPG), and Dose-Response Parameters in Eight Patients with NIDDM and Eight Control Subjects. (Calculation of $R^2$, a Measure of Curve-Fitting Precision, is Described in the Text)*

| | Age | IBW | FPG | $AIR_{max}$ | $PG_{50}$ | K | $R^2$ |
|---|---|---|---|---|---|---|---|
| | *yr* | *%* | *mg/dl* | *μU/ml* | *mg/dl* | | |
| Controls | | | | | | | |
| Subject 1 | 47 | 133 | 93 | 871 | 211 | 2.03 | >99.9 |
| 2 | 40 | 147 | 93 | 608 | 182 | 3.89 | >99.9 |
| 3 | 62 | 117 | 92 | 202 | 173 | 2.83 | 99.8 |
| 4 | 52 | 132 | 87 | 428 | 175 | 2.64 | >99.9 |
| 5 | 44 | 121 | 92 | 156 | 205 | 2.41 | 99.4 |
| 6 | 44 | 120 | 97 | 203 | 98 | 2.76 | 98.4 |
| 7 | 38 | 139 | 94 | 723 | 232 | 2.67 | >99.9 |
| 8 | 41 | 124 | 93 | 407 | 297 | 3.52 | 99.6 |
| Mean | 46 | 129 | 93 | 450 | 197 | 2.84 | |
| SEM | 3 | 4 | 1 | 93 | 20 | 0.21 | |
| Diabetics | | | | | | | |
| Subject 1 | 53 | 139 | 129 | (109)* | * | * | |
| 2 | 38 | 137 | 312 | 22 | 325 | 1.89 | 44.1 |
| 3 | 50 | 141 | 235 | 116 | 233 | 2.47 | 97.5 |
| 4 | 41 | 146 | 253 | 144 | 258 | 2.54 | 98.8 |
| 5 | 52 | 123 | 166 | 68 | 110 | 3.27 | 98.6 |
| 6 | 54 | 118 | 244 | 39 | 218 | 5.07 | 64.5 |
| 7 | 69 | 123 | 333 | 32 | 182 | 2.15 | 59.3 |
| 8 | 51 | 123 | 184 | 159 | 313 | 1.90 | 98.8 |
| Mean | 51 | 131 | 232 | 83 | 234 | 2.76 | |
| SEM | 3 | 4 | 25 | 21 | 28 | 0.42 | |

* These parameters could not be estimated for subject 1 by curve fitting. The insulin response at a glucose level of 661 mg/dl, 109, is listed.

*Table II. A Comparison of Plasma Levels of Glucose, Norepinephrine, and Epinephrine in Control and Diabetic Subjects*

| | Plasma glucose level | Norepinephrine | Epinephrine |
|---|---|---|---|
| | *mg/dl* | *pg/ml* | *pg/ml* |
| Controls ($n$ = 8) | | | |
| First study | | | |
| Initial | 96±2 | 241±38 | 28±7 |
| After 2 h hiatus | 88±2 | 276±44 | 31±5 |
| Final | 612±22 | 328±52 | 25±5 |
| Second study | | | |
| Initial | 94±2 | 286±43 | 35±7 |
| After 2 h hiatus | 91±2 | 306±46 | 34±8 |
| Final | 462±12 | 358±67 | 39±5 |
| Diabetics | | | |
| First study | | | |
| Before insulin ($n$ = 7) | 242±26 | 334±83 | 54±9 |
| After insulin ($n$ = 7) | 119±10 | 370±49 | 136±35* |
| Final ($n$ = 8) | 623±31 | 219±20 | 58±15 |
| Second study ($n$ = 8) | | | |
| Initial | 241±7 | 298±38 | 58±12 |
| Final | 461±18 | 245±37 | 60±7 |

* $P < 0.05$, compared with epinephrine before insulin.

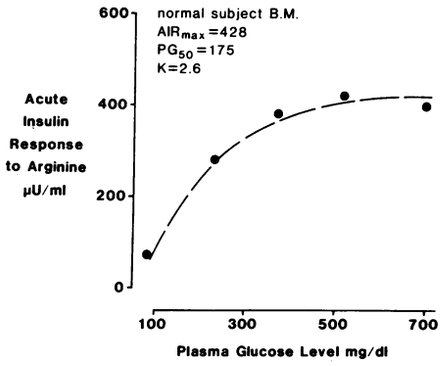


*Figure 4.* By use of the curve fitting process described in the text, a curve (dashed line) was fitted to insulin response data in order to estimate the asymptote approached as a maximum ($AIR_{max}$). Data from one representative normal subject are depicted.

response curve were compared. The slope of the fourth segment was less than the slope of the third segment in seven of eight control subjects and in seven of the eight diabetic patients, which suggests that insulin responses consistently began to level off over the glucose range from 345 to 615 mg/dl in controls and diabetics. The overall shape of the curves relating prestimulus insulin level to glucose level are similar. (Using the same method, prestimulus insulin levels began to level off in six of eight control subjects and in all eight diabetic patients.)

The modified Michaelis-Menten equation was employed to estimate the maximal acute insulin response, the plasma glucose level at half maximum, and the $K$ exponent for each control and each diabetic subject. Individual parameters for controls and diabetics are given in Table I. As can be appreciated from the representative example of a control illustrated in Fig. 4, the curve predicted by this equation closely approximates the actual insulin response data. $AIR_{max}$ and $PG_{50}$ values for all control and seven of the eight diabetic subjects, as estimated by such curve fitting, are compared in Fig. 5. Parameters for one diabetic patient (subject 1, Table I) could not be estimated using the curve-fitting process because his insulin responses did not approach a maximum over the glucose range tested. Almost complete separation in $AIR_{max}$ values was observed between control subjects (range: 156–871 μU/ml, mean 450±93 μU/ml) and diabetic patients (range: 68–159 μU/ml, mean:

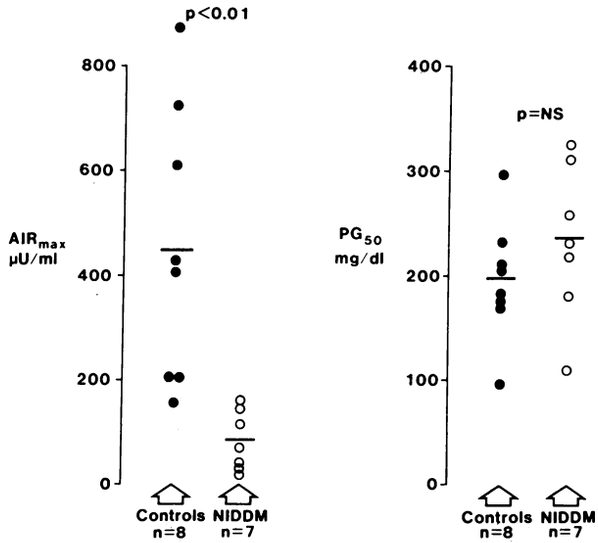


*Figure 5.* A comparison of $AIR_{max}$ and $PG_{50}$ in eight control subjects (solid circles) and seven patients with NIDDM (open circles). Horizontal bars indicate mean values. NS, not significant.

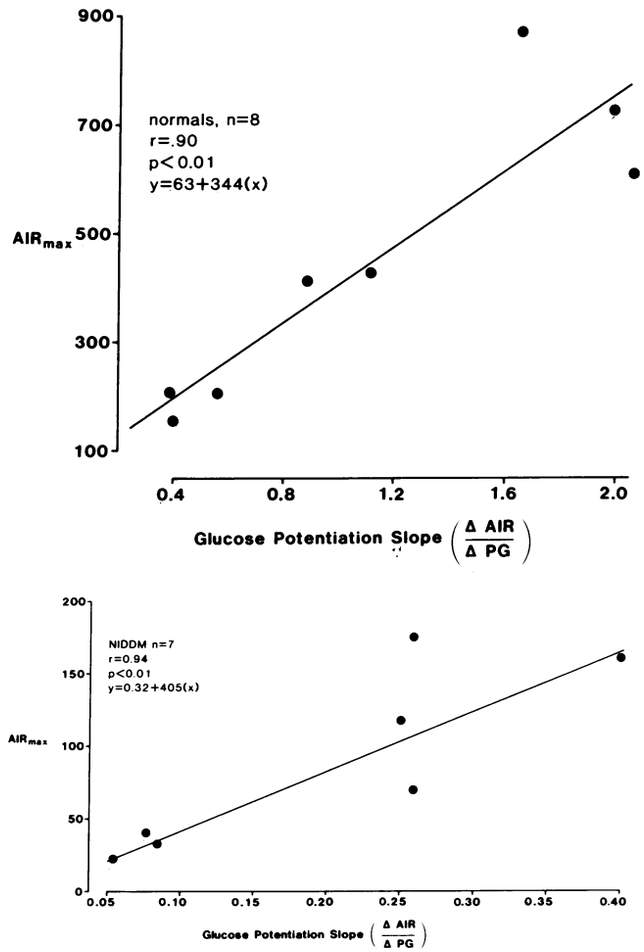


*Figure 6.* Regression analysis comparing $AIR_{max}$ (estimated from curve fitting of the dose-response data) and glucose potentiation slope (Δ acute insulin response [AIR]) ÷ (Δ plasma glucose [PG]) calculated from data at the two lowest PG levels in eight normal subjects (*A*) and in seven patients with NIDDM (*B*).

83±21 μU/ml, $P < 0.01$). In contrast, $PG_{50}$ values for control subjects (range: 98–297, mean; 197±20 mg/dl) were similar to, and overlapped considerably with those of diabetic patients (range: 110–325, mean: 234±28 mg/dl, $P$ not significant). $K$ exponents were also similar in both groups (control: 2.66±0.21, diabetics: 2.76±0.43, $P$ not significant). Estimates of curve-fitting precision ($R^2$) are provided for normal subjects and for diabetic patients in Table I.

In order to assess whether the single insulin response to arginine at a plasma glucose level of ~615 mg/dl provided an estimate of maximum response similar to the $AIR_{max}$ value estimated by curve fitting, each subject's insulin response at the highest glucose level was compared with that subject's $AIR_{max}$. A highly significant correlation between these two estimates of the maximal response was apparent in controls ($r = 0.999$, $P < 0.001$) and in diabetics ($r = 0.977$, $P < 0.001$). $AIR_{max}$ was also highly correlated with glucose potentiation slope (which we have used previously as an index of B cell responsiveness to glucose level [2]) both in control subjects ($r = 0.90$, $P < 0.01$) (Fig. 6 *A*) and in diabetic patients ($r = 0.94$, $P < 0.01$) (Fig. 6 *B*).

To determine if the magnitude of $AIR_{max}$ was associated with degree of adiposity, $AIR_{max}$ was plotted as a function of percentage of ideal body weight in control and diabetic subjects (Fig. 7). A significant correlation was observed in controls ($r = 0.78$, $P < 0.05$) but not in patients with NIDDM ($r = 0.37$, $P$ not significant).

*Glucagon secretion.* Acute glucagon secretory responses to arginine were also obtained at five different plasma glucose levels (Fig. 8). Glucagon responses obtained in control subjects at euglycemia before the 2-h glucose washout period were similar to those obtained after this period (101±13 vs. 94±12 pg/ml, $P$ not significant). Glucagon responses were higher in diabetic patients throughout the glucose range ($P < 0.05$ at all levels), and prestimulus glucagon levels (not shown) were significantly higher in diabetic patients at all levels except at the glucose level of 461 mg/dl.

The mean glucagon response appeared to reach a minimum at a plasma glucose level of ~460 mg/dl in the control group.

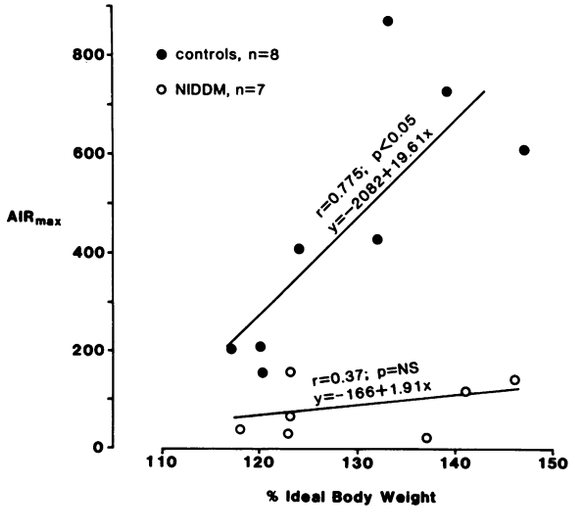


*Figure 7.* Regression analysis comparing $AIR_{max}$ and percentage of ideal body weight (measure of adiposity) in eight normal subjects and in seven patients with NIDDM. NS, not significant.

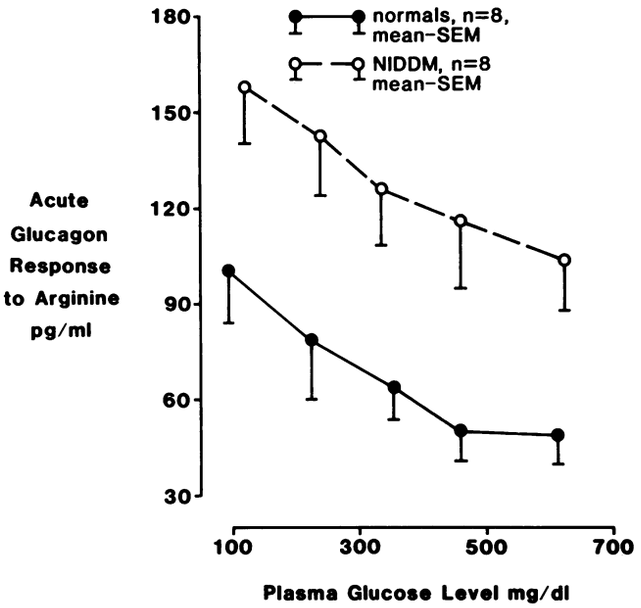


*Figure 8.* A comparison of acute glucagon responses to arginine as a function of glucose level in eight normal subjects and in eight patients with NIDDM.

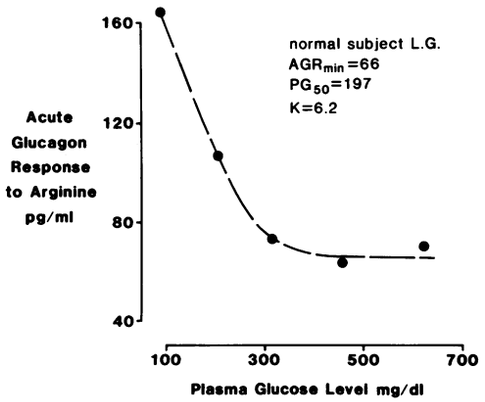


*Figure 9.* By use of the modified Michaelis-Menten equation described in the text, a curve (dashed line) was fitted to glucagon response data in order to estimate the asymptote approached as a minimum ($AGR_{min}$). Data from one representative normal subject is depicted.

Further, the slope of the glucagon response segment over the glucose range 345–460 mg/dl was steeper than the slope of the segment from 460 to 615 mg/dl in seven of the eight control subjects. Because the glucagon responses in control subjects appeared to approach a minimum in the high plasma glucose range, a minimum acute glucagon response ($AGR_{min}$), $PG_{50}$, and $K$ value were estimated for seven of the eight control subjects, as exemplified for one subject in Fig. 9. Dose-response parameters could not be estimated for one subject by use of the curve-fitting process. In the control group, $AGR_{min}$ averaged 38±11 pg/ml, $PG_{50}$ averaged 371±83 mg/dl, and $K$ averaged 4.6±0.7.

In contrast to the control group, glucagon responses to arginine in the diabetic group declined continuously as glucose levels increased, without approaching a minimum (Fig. 8). Moreover, the slope of the glucagon response segment over the glucose range 330–461 mg/dl was steeper than the slope of the segment from 461 to 623 mg/dl in only two of eight diabetic individuals ($P = 0.02$ as compared with controls, Fisher's Exact Test). Because there was no indication that glucagon responses approached an asymptote as the glucose level increased in diabetic patients, it was not appropriate to apply the curve-fitting model to estimate $AGR_{min}$ in diabetics.

*Catecholamines.* Norepinephrine and epinephrine values for control and diabetic subjects are given in Table II. On both study days in control subjects and on the second study day in diabetics, no significant change in catecholamine levels from initial values was observed. However, on the first study day in diabetics, the plasma epinephrine level increased from 54±9 before the insulin infusion to 136±35 pg/ml 1 h after discontinuing the insulin ($P < 0.05$). Plasma glucose levels, which were measured every 20 min during the insulin infusion and during the insulin washout period, fell to a mean nadir of 101±8 mg/dl (Fig. 2 *A*). A glucose nadir below 87 mg/dl (76 mg/dl) was observed in only one patient. Thus, the plasma glucose levels did not fall into the range which is usually considered hypoglycemic.

## Discussion

When compared with a control group of similar mean age and adiposity, patients with NIDDM demonstrated lower acute insulin secretory responses to arginine at all five matched plasma glucose levels tested (from ~110–615 mg/dl). Mean insulin responses of both groups and insulin responses of nearly all individuals in both groups approached a maximum (an asymptote) over the upper portion of this glucose range. Both by inspection of dose-response curves that were derived from mean data of both groups (Fig. 3 *B*) and by estimation of each individual's $AIR_{max}$ (Fig. 5), it is clear that the maximum insulin responsiveness to the potentiating effects of glucose in diabetic patients is greatly diminished. Prestimulus insulin levels obtained after each 30-min glucose clamp, which reflect direct glucose level-induced second-phase insulin secretion, also appeared to approach a much lower maximum in diabetic than in control subjects (Fig. 3*A*). Thus, it appears that both maximal responsiveness to glucose as a potentiator of insulin responses to arginine and maximal responsiveness to a sustained glucose level directly are decreased in NIDDM. These data suggest that neither insulin responses to nonglucose stimuli nor second-phase insulin responses to glucose can be normalized at any glucose level in patients with NIDDM who have marked fasting hyperglycemia.

The observation of diminished maximal insulin responsiveness to glucose level in NIDDM suggests the presence of a reduced insulin secretory capacity in these patients. Such a diminished secretory capacity could have several causes, one of the most obvious of which is a reduced islet B cell mass. Postmortem studies of patients with NIDDM have revealed a reduction in B cell mass to ~50% of normal (21–23), although B cell mass is difficult to measure precisely because of amyloid and glycogen deposition and postmortem changes. A 50% reduction in B cell mass is unlikely to be the sole cause of hyperglycemia in NIDDM, however, in view of the observation that pancreatectomies of <70% seldom lead to permanent fasting hyperglycemia (24, 25). Therefore, this reduction of secretory capacity is probably also due in part to a generalized functional B cell defect, such as an impairment of proinsulin biosynthesis or insulin emiocytosis.

In contrast to the large differences in $AIR_{max}$ between diabetic and control subjects, no major differences in $PG_{50}$ or in the $K$ exponent were observed. Such findings indicate that diabetics and euglycemic control subjects increase their responsiveness to the potentiating effects of glucose level and achieve a maximum response over a similar plasma glucose range. The finding of normal $PG_{50}$ values in patients with NIDDM suggests that a glucose-sensing abnormality is not likely to be the major cause of impaired regulation of insulin secretion by glucose. A reduction in glucose-sensing units (if spare units exist, as in many hormone receptor systems [26]) would be expected to lead to an elevated $PG_{50}$ rather than a decreased $AIR_{max}$. $PG_{50}$ values varied over quite a large range in diabetics (110–325) and controls (98–297 mg/dl). Since $PG_{50}$ values did not correlate with age, percentage of ideal body weight, or fasting plasma glucose level in either group (data not shown), the cause for such variation is unclear. Because of such variability in $PG_{50}$ values, a small but real difference in $PG_{50}$ between patients with NIDDM and nondiabetic subjects cannot be excluded with certainty.

Although a reduction of $AIR_{max}$ in diabetic patients could result from an islet abnormality, it is less likely to result from primary insulin resistance. The positive correlation between a measure of adiposity and $AIR_{max}$ (Fig. 7) in nondiabetic control subjects suggests that B cells normally adapt and compensate for the insulin resistance of obesity by increasing responsiveness to glucose. This finding agrees with the reports of Karam et al. (27) and Beard et al. (28) that B cell responsiveness to glucose level is increased in obese subjects,

and suggests that tissue insensitivity to insulin normally leads to an increase in insulin secretory capacity. Thus, it is unlikely that the marked impairment of B cell secretory capacity which we observed in patients with NIDDM is secondary to the presence of insulin resistance in these patients.

Other workers have also investigated the relationship between glucose level and insulin responses in patients with NIDDM. In contrast to our results, Cerasi et al. (3) found that second-phase insulin responses to hyperglycemia in normal persons did not appear to approach a maximum asymptote with increasing hyperglycemia. However, in this study, insulin responses were measured at glucose levels of 348 and 549 mg/dl, but not between. Thus, it is possible that a second-phase insulin response obtained at a plasma glucose level of ~400 mg/dl might have been similar to the response obtained at 549 mg/dl. Although Cerasi et al. (3) reported that insulin responses of "mild diabetics" also failed to approach a maximum at hyperglycemic levels, the fasting blood glucose concentration of these patients averaged <100 mg/dl. Thus, these patients would probably not be considered diabetic by present standards (29).

Botha et al. (30) also examined the relationship between glucose and insulin responses in man, but they examined insulin responses at much lower blood glucose levels than the levels examined in the present study or in the study of Cerasi et al. (3). Although second-phase insulin secretion did not appear to approach a maximum in normal or in diabetic subjects, maximal blood glucose levels achieved were only ~160 mg/dl in normals and 265 mg/dl in diabetics. Thus, it is quite possible that second-phase responses would have approached a maximum if blood glucose level had been raised to the 400–600 mg/dl range. In addition, a glucose washout period was not present between the different levels of hyperglycemia during which insulin responses were determined in normal subjects in the study of Botha et al. (30). Thus, in view of the effect of hyperglycemia to augment subsequently measured glucose-induced insulin responses (10, 11), responses at high glucose levels may have been distorted by preceding hyperglycemia.

The relationship between glucose and insulin responses which we have observed in man is similar to that in normal rat islet tissue in vitro. Insulin release appears to reach a near maximum at a glucose level of 300–400 mg/dl in the perfused rat pancreas (31) and in isolated rat islets (32, 33). In addition, the glucose level at half-maximal insulin output in rat islets, 160–180 mg/dl (33), is similar to the $PG_{50}$ for normal humans in the present study, which is 197±20 mg/dl. The close agreement with in vitro studies suggests that other neuroendocrine or metabolic changes that may have occurred in vivo during these studies did not have a major influence on the regulation of insulin secretion by glucose.

During these in vitro studies (31, 33), the ambient glucose was maintained at a normal level before measuring insulin output at elevated glucose levels in order to avoid glucose priming effects. Similarly, in the present study, 2-h glucose washout periods followed hyperglycemic clamp experiments in normal subjects whenever an additional hyperglycemic clamp was to be performed later on the same day. Insulin and glucagon responses to arginine that were obtained at euglycemia after the glucose washout period were similar to the responses that were obtained at euglycemia before the glucose washout period. Thus, it appears that 2 h is a sufficient length of time to resolve any possible priming effect of moderate hyperglycemia (224–355 mg/dl) on B and A cell secretory responses.

In addition to estimation of $AIR_{max}$ by curve fitting, we have examined two other methods of assessing islet responsiveness to glucose level. The first, the glucose potentiation slope, is the change in acute insulin response to arginine per unit change in glucose level in the linear (low) range of the dose-response-curve, and has been utilized as a measure of B cell responsiveness to glucose level (2). The glucose potentiation slope is highly correlated with $AIR_{max}$ both in normal subjects and in diabetics (Fig. 6). It thus appears that glucose potentiation slope can be employed as an indirect estimate of B cell secretory capacity. We have also compared the single acute insulin response measured at a glucose level of ~615 mg/dl with the $AIR_{max}$ estimated by curve fitting. An extremely tight correlation exists between these two estimates of secretory capacity in normal subjects and in diabetics. Consequently, it appears that a single determination of the acute insulin response to arginine at very high plasma glucose levels (>500 mg/dl) can also serve as an alternative to measuring a series of insulin responses throughout the dose-response range when one desires to estimate glucose-modulated B cell secretory capacity. Of the various methods of estimating insulin secretory capacity, measurement of a single insulin response to arginine at a glucose level of ~600 mg/dl is probably the simplest to perform and was well tolerated by all subjects in this study.

We also examined glucagon secretory responses to arginine over a wide range of plasma glucose levels. We found that glucagon responses to arginine were significantly higher in the diabetic patients than in controls at all five matched glucose levels. A trend toward higher glucagon responses in patients with NIDDM than in controls compared at three matched glucose levels has previously been reported (34). Our study was not designed to ascertain whether such an increase in glucagon responses in patients with NIDDM is a result of primary A cell hyperfunction or is secondary to impaired insulin secretion.

In addition to a difference in magnitude of glucagon responses, patients with NIDDM demonstrated a different pattern of suppression by hyperglycemia. Mean glucagon responses to arginine in the normal control subjects are nearly maximally suppressed at a mean glucose level of 462 mg/dl, which is the same degree of hyperglycemia necessary to maximally stimulate the acute insulin response to arginine. In contrast, glucagon responses to arginine in diabetics declined continuously throughout the plasma glucose range from 122±7

to 623±31 mg/dl without approaching a minimum. The relationship between acute glucagon response and plasma glucose level is very nearly linear throughout this range and the slope of the final curve segment is greater than that of the preceding segment in only two of eight diabetic patients. Thus, the evidence suggests that greater degrees of hyperglycemia than were tested in this study would be required to maximally suppress the glucagon response to arginine in NIDDM patients. Although specific glucagon response parameters could not be measured in patients with NIDDM, it thus appears that A cells in these patients may be less sensitive (i.e., higher $PG_{50}$) to the inhibitory effects of hyperglycemia.

Norepinephrine and epinephrine levels were also measured before and after the insulin infusion and at other times during the studies in controls and diabetics (Table II). The only significant change in catecholamine levels in controls or diabetics occurred after the insulin infusion in diabetics and consisted of a increase in epinephrine levels from 54±10 pg/ml before the insulin infusion at a mean plasma glucose level of 242±26 mg/dl to 136±35 pg/ml at a mean plasma glucose of 119±10 mg/dl ($P < 0.05$, paired $t$ test). As previously observed (35, 36), such a release of epinephrine suggests that in chronically hyperglycemic persons, a rapid lowering of glucose levels may cause counterregulatory hormone release even though glucose levels are not in the range that is usually considered hypoglycemic. We employed the multiple regression model of Beard et al. (37) to estimate the potential effect of the observed epinephrine rise to inhibit the insulin response to arginine. Such a method predicts that if no increase in epinephrine had occurred, the mean insulin response to arginine at a glucose level of 119 mg/dl would have been 25 instead of 17 $\mu$U/ml. Recalculation of $AIR_{max}$, $PG_{50}$, and $K$ using such a correction does not lead to appreciable changes in these dose-response parameters. Thus, this rise in epinephrine is not likely to have significantly altered our primary results and conclusions.

In summary, patients with NIDDM possess markedly diminished maximal insulin responsiveness to the potentiating effects of glucose, as compared to nondiabetic controls of similar age and adiposity. In contrast, the glucose levels at half-maximal responsiveness were similar in the two groups. Taken together, these findings do not support the presence of a glucose-sensing defect in NIDDM. Rather, patients with NIDDM appear to have a reduction in insulin secretory capacity. The exact nature of this B cell lesion remains to be elucidated.

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