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# Dose-Kinetics of Pancreatic Glucagon Responses to Arginine and Glucose in Subjects with Normal and Impaired Pancreatic B Cell Function

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**Summary.** Pancreatic glucagon responses to different amounts of intravenous arginine and glucose were studied in 10 insulin-dependent diabetics, 14 healthy controls (high insulin responders) and 15 subjects with decreased insulin response to glucose but normal intravenous glucose tolerance (low insulin responders). The dose-kinetics of the glucagon response was studied by using four different arginine doses. The suppressive effect of glucose was evaluated by infusing three glucose doses during a submaximal stimulation with arginine. The diabetics were tested first when under fair metabolic control and then following intensive treatment with insulin to produce near-normalisation of blood glucose. Finally, five subjects underwent insulin-induced hypoglycaemia. The changes in plasma glucagon and blood α-amino-nitrogen in response to the four arginine doses were significantly correlated in all groups but the slope of the dose response curve was steeper in the poorly controlled-diabetics than in the non-diabetics. These diabetics displayed higher fasting plasma glucagon values than healthy controls (high insulin responders) ($224 \pm 4$ versus $151 \pm 22$ pg/ml, $p < 0.01$), higher plasma glucagon responses to arginine and an absence of inhibition by glucose of the arginine-stimulated glucagon release. In strictly controlled diabetic patients, fasting plasma glucagon levels ($176 \pm 16$ pg/ml) were not significantly different from healthy controls, the glucagon response to arginine returned to the normal range, A cell suppressibility by glucose was restored and A cell stimulation by hypoglycaemia reappeared. In the low insulin responders, fasting plasma glucagon was not different from that of high responders ($107 \pm 12$ pg/ml), the slope of the dose response curve to arginine was similar in both groups and the A cells were inhibited by glucose to a similar extent. These results support the concept that islet A cell dysfunction in diabetes is not a primary phenomenon.

**Key words:** Glucagon, A cell, low insulin responders, diabetes.

The cause of the islet A cell abnormalities and their importance in the pathophysiology of diabetes mellitus remains controversial [1–5]. It has been suggested that A cell dysfunction, which is a secondary phenomenon in experimental diabetes, might be a primary defect in human diabetes [1]. If this hypothesis is correct, A cell dysfunction might be present in potentially diabetic subjects before the establishment of overt metabolic disturbances. On the contrary if hyperglucagonaemia is induced by the metabolic changes of diabetes, A cell function should be normal in overt diabetics in whom strict control of their disease is achieved. This has recently been demonstrated in some groups of aggressively treated patients [5–8].

In the present study islet A cell function has been evaluated using various doses of arginine and glucose in the following groups of patients. First, low insulin responders with a normal glucose disappearance but decreased insulin response to glucose infusion who have previously been demonstrated to have a higher probability of developing diabetes [9, 10]. Second, insulin dependent diabetics before and after strict glycaemic control and third, normal healthy subjects.

## Subjects and Methods

### *Subjects*

The characteristics of the subjects are shown in Table 1. Sex distribution, age range and percentage ideal body weight were similar in all groups. No subject was obese. The non-diabetic subjects were classified as high or low insulin responders before the present study according to their insulin response to glucose infusion [9, 10]. The K value of the IV glucose tolerance test was above 1.0 in all subjects.

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**Table 1.** Characteristics of the subjects studied

| Group | Age (years) | Ideal body weight (%) | K value (%/min) | Duration of diabetes (years) |
|---|---|---|---|---|
| High insulin responders ($n = 14$) | 32 ± 2 | 92 ± 3 | 2.7 ± 0.2 | – |
| Low insulin responders ($n = 15$) | 37 ± 2 | 102 ± 1 | 1.6 ± 0.1[a] | – |
| Diabetics ($n = 10$) | 28 ± 3 | 101 ± 2 | – | 10 ± 2 |

Results are presented as mean ± SEM. [a] statistical significance of differences with the high insulin responders: $p < 0.01$. Numbers of subjects are indicated in parentheses. % ideal body weight was calculated according to Statist Bull Metrop Life Insur Co [1959]

However, the low insulin responders presented significantly lower K values than the high responders as has previously been demonstrated in a larger group of subjects: 1.6 ± 0.1 versus 2.7 ± 0.2%/min [10].

All diabetic patients had been ketotic on admission and were insulin-dependent. Renal and hepatic function was normal as assessed by serum creatinine and alkaline phosphatase concentrations. No subjects were taking any medication other than insulin at the time of study. None had other chronic disease. The patients were tested first while on a single dose of Lente insulin (25–40 U), providing fair control of glycaemia. The blood glucose was 8.2 ± 1.3 mmol/l fasting and 13.7 ± 1.3 mmol/l 2 h after lunch. A second test was performed after one week of rigid control with Isophane insulin (20–34 U at 08.00 h and 10–34 U at 20.00 h). The blood glucose was 4.3 ± 0.6 mmol/l fasting and 6.9 ± 1.3 mmol/l 2 h after lunch (values collected on seven different days). In addition a slow IV infusion of insulin (Actrapid 1.7 U/h) was maintained for the 12 h preceding and during the test in order to maintain blood glucose levels close to normal values.

### *Investigations*

All tests were performed with the informed consent of the subjects and in accordance with the principles of the Declaration of Helsinki.

Arginine HCl (Vitrum, Stockholm, Sweden) was administered IV as a rapid injection followed by a constant rate infusion for 30 min. Four doses were used: 1) 0.12 mmol/kg body weight as a bolus, followed by an infusion of 0.01 mmol · kg$^{-1}$ · min$^{-1}$; 2) 0.24 mmol/kg bolus and 0.02 mmol · kg$^{-1}$ · min$^{-1}$ infusion; 3) 0.72 mmol/kg bolus and 0.04 mmol · kg$^{-1}$ · min$^{-1}$ infusion; 4) 1.08 mmol/kg bolus and 0.07 mmol · kg$^{-1}$ · min$^{-1}$ infusion. These tests are referred to below as arginine I, II, III and IV respectively.

Pancreatic A cell inhibition was studied by combining glucose administration with arginine III. The following glucose doses were used: 0.55 mmol · kg$^{-1}$ · min$^{-1}$ for 30 min; 1.38 mmol/kg bolus and 0.05 mmol · kg$^{-1}$ · min$^{-1}$; 2.75 mmol/kg bolus and 0.11 mmol · kg$^{-1}$ · min$^{-1}$. These are designated as glucose I, II, and III, respectively.

The four arginine doses were tested in 14 high insulin responders, 10 low insulin responders and 10 diabetics. The glucose + arginine tests were performed in five of the high responders and five of the low responders. In the 10 diabetics, A cell stimulation by arginine III and inhibition by glucose III were performed before and during optimised control. Finally, five of these diabetics underwent insulin-induced hypoglycaemia, obtained by IV infusion of 20 U Actrapid over 30 min.

All tests were performed in the supine position at 8.00–10.00 h after an overnight fast. At least one week elapsed between consecutive investigations. Venous blood samples were collected into chilled heparinised tubes, handled appropriately for further assays (see below) and then centrifuged at +4 °C. The supernatants were stored at −20 °C until processing. Glucose was measured in whole blood by a glucose oxidase method [11]; $\alpha$-amino-nitrogen ($\alpha$-$NH_2$) by the cuprizone method [12] after deproteinisation with 5% (w/v) trichloracetic acid (0.5 ml for 0.1 ml blood). Alanine was determined by an enzymatic fluorimetric method [13] after deproteinisation of 0.5 ml blood with 0.5 ml 7.5% (w/v perchloric acid). Aprotinin (2,000 U/l ml blood) was added to the samples intended for hormone assay. Plasma immunoreactive glucagon (IRG) and insulin were determined by radioimmunoassay, using antiserum 30 K for glucagon (Professor R. H. Unger, Dallas, Texas) without prior extraction of plasma samples [14].

The lowest amount of glucagon standard which gave a significant variation from the zero standard of the bound/free ratio was 7 pg/ml, corresponding in whole plasma to a lowest detectable value of 21 pg/ml. Interassay variation was less than 5%.

Results are presented as mean ± SEM for each time point. The sum of the glucagon values during individual experiments was calculated to give the integrated glucagon response for 90 min. Similarly the sum of the substrate variations from basal values was calculated in each individual experiment to give the net increase or decrease of blood glucose, $\alpha$-$NH_2$ and alanine.

Standard statistical methods were used for analysis of results. Non-parametric statistics were used for comparisons of the summed (integrated) responses and of the sums of variations from basal values [15].

## Results

### *Basal Values*

Basal values were calculated for each subject by taking the mean of individual measurements on five to eight different occasions. In non-diabetic subjects there was no significant difference between high and low insulin responders with respect to fasting blood glucose levels (4.2 ± 0.1 versus 4.3 ± 0.1 mmol/l); $\alpha$-$NH_2$ (5.3 ± 0.2 versus 5.9 ± 0.2 mmol/l); alanine (359 ± 29 versus 361 ± 40 mmol/l) and insulin (24.0 ± 0.7 versus 24.0 ± 1.4 mU/l). Plasma immunoreactive glucagon (IRG) levels were not significantly different between low insulin responders and the high responders (107 ± 12 versus 151 ± 22 pg/ml). The diabetic patients differed significantly from the two other groups for fasting blood glucose (9.2 ± 0.3 mmol/l); $\alpha$-$NH_2$ (4.8 ± 0.2 mmol/l) and plasma IRG (224 ± 4 pg/ml). During the excellent control of the diabetics the differences for glucose (4.3 ± 0.02 mmol/l) and IRG (176 ± 16 pg/ml) disappeared, but not for $\alpha$-$NH_2$ (4.7 ± 0.2 mmol/l). Blood alanine was not different from the other groups (362 ± 18 μmol/l).

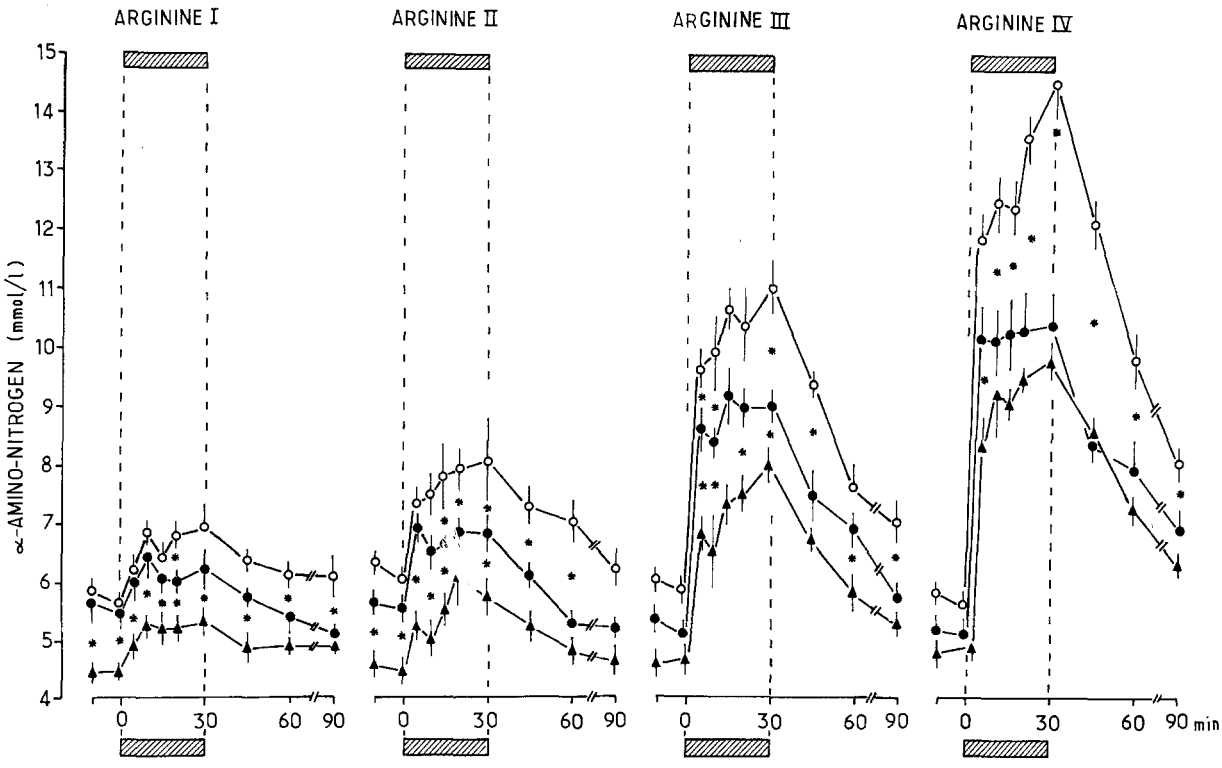


**Fig. 1.** Blood $\alpha$-amino-nitrogen during arginine infusions in high insulin responders (●), low insulin responders (○) and poorly controlled diabetics (▲); asterisks indicate $p < 0.05$ or lower from the corresponding value in high responders. The hatched bars indicate the duration of arginine infusion. (see text for the four arginine doses)

**Table 2.** Blood glucose: basal values and increases over basal during arginine and arginine + glucose infusions in control and diabetic subjects

| Groups | Basal values (mmol/l) | Sum of increases over basal ($mmol/l^{-1} \cdot 90\ min^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Arginine I | Arginine II | Arginine III | Arginine IV | Arginine III + glucose I | Arginine III + glucose II | Arginine III + glucose III |
| *Control subjects* | | | | | | | | |
| High responders | 4.2 ± 0.1 | 0.4 ± 0.6 | 1.6 ± 0.4 | 0.8 ± 0.7 | 1.0 ± 0.9 | 10.4 ± 0.8 | 19.5 ± 0.5 | 55.0 ± 2.0 |
| Low responders | 4.3 ± 0.1 | 1.1 ± 0.4 | 2.5 ± 0.6 | 1.9 ± 0.7 | 1.9 ± 0.4 | 9.2 ± 0.8 | 38.0 ± 1.1[b] | 80.0 ± 6.5[b] |
| *Diabetic subjects* | | | | | | | | |
| Fair control | 9.3 ± 0.3[b] | 8.4 ± 2.7[a] | 8.8 ± 3.9[a] | 19.6 ± 2.7[b] | 26.4 ± 3.0[b] | – | – | 130.1 ± 5.0[b] |
| Good control | 4.3 ± 0.7 | – | | 5.5 ± 3.6 | – | – | – | 45.5 ± 3.5 |

Increases over basal represent the algebraic sums of variations from basal values (i. e. the mean of time samples −15 and zero min) as calculated from time samples +5, 10, 15, 20, 45, 60 and 90 min. Results are presented as mean ± SEM. Statistical significance of differences with high insulin responders [a] $p < 0.01$; [b] $p < 0.001$

*Arginine Infusion Tests*

In general, during the arginine loadings higher blood $\alpha$-$NH_2$ values were observed in the low-insulin responders and lower values in the diabetics when compared with the high insulin responders (Fig. 1). The magnitude of the increase was similar in all groups for any given increase in the dose of arginine used.

In high insulin responders, blood glucose displayed the usual slight rise followed by a minor decrease. The rise was more pronounced in low responders and even more so in the diabetics (Table 2).

Plasma IRG rose proportionally to the arginine dose in all groups, even with the lowest infusion rate (Fig. 2). With the two lowest arginine doses, peak values were reached at 5 min, with arginine III and IV at 30 min. Plasma IRG returned to baseline at 90 min or earlier, except in the case of arginine IV. In the diabetic patients plasma IRG was always higher than in non-diabetic subjects. The differences between dia-

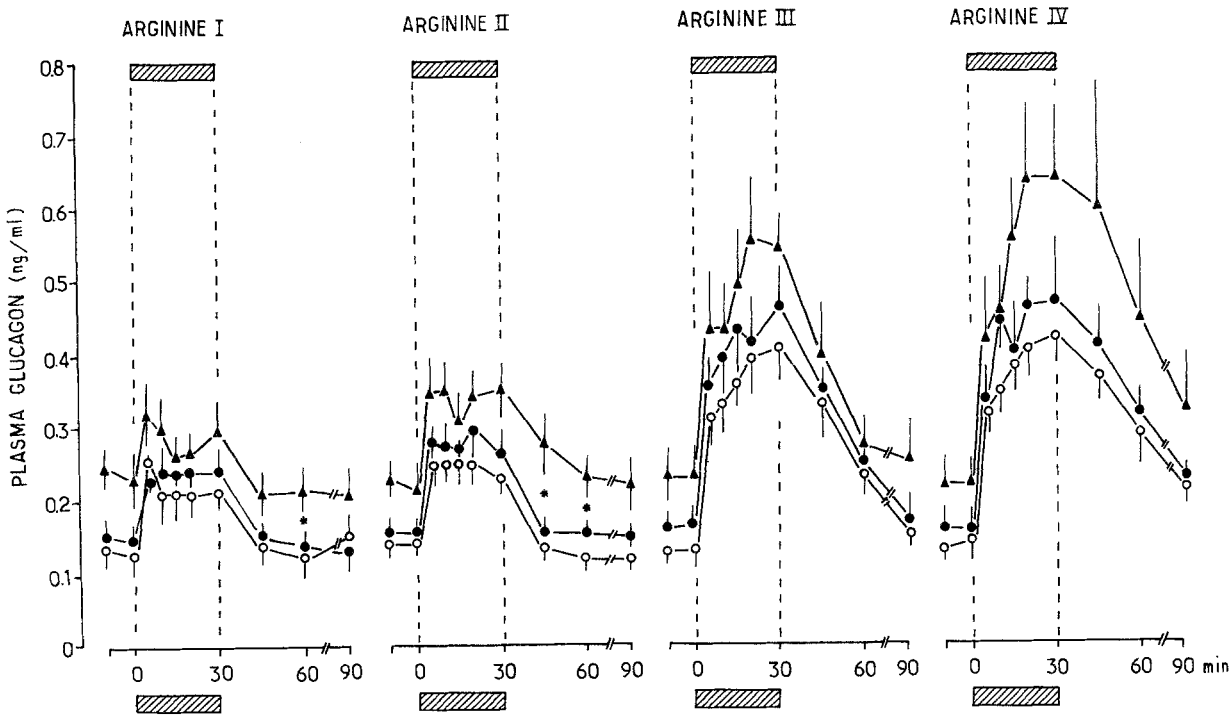


**Fig. 2.** Plasma glucagon variations during arginine infusions in high insulin responders (●), low insulin responders (○) and poorly controlled diabetics (▲). Results are presented as in Fig. 1

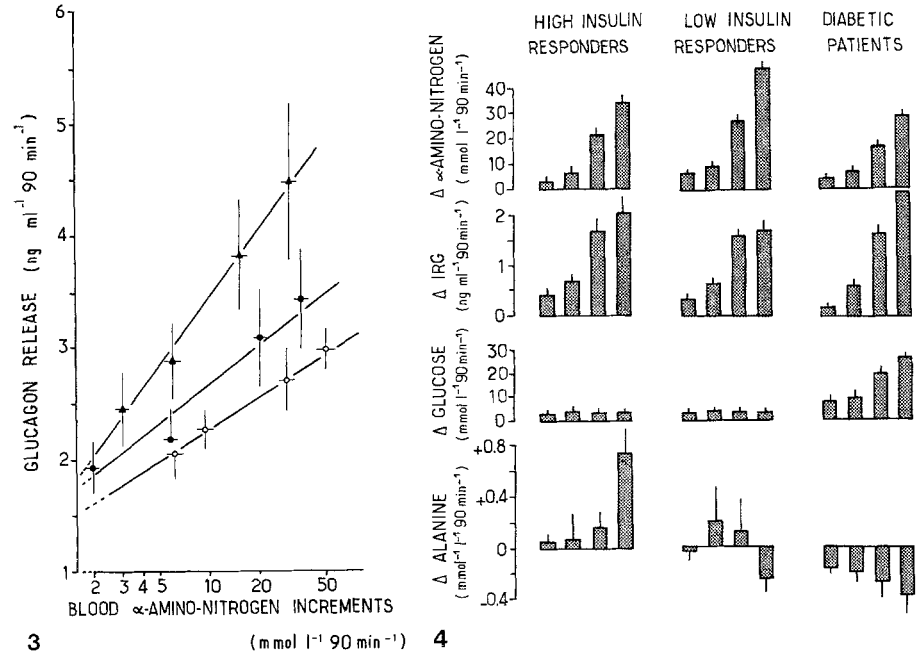


**Fig. 3.** Dose-response curves relating the glucagon responses (sum of glucagon values for 90 min) to the corresponding $\alpha$-amino-nitrogen increases in high insulin responders (●), low insulin responders (○) and diabetics (▲). Results are presented as mean ± SEM. Equations for dose response curves were: $y = 1.29 \log \times + 1.34$ in high insulin responders ($n = 14$); $y = 1.20 \log \times + 1.03$ in low insulin responders ($n = 10$); $y = 2.08 \log \times + 1.30$ in diabetics ($n = 10$)

**Fig. 4.** Algebraic sum of variations from basal values of $\alpha$-amino-nitrogen, glucagon (IRG), glucose and alanine during the 90 min of the tests. Algebraic sum of variations from basal was calculated as in Tables 1 and 2. The four columns in each group of results correspond to the four doses of arginine (see text for details on the arginine infusions)

betics and high insulin responders were significant at a few time-points. On the other hand, comparisons between diabetics and low insulin responders yielded significant differences at almost all sampling times. No significant differences were observed between high and low responders, but IRG response curves were, in most instances, lower in the low responders than in high responders.

There was a linear relationship between the integrated IRG response over 90 min and the log of $\alpha$-$NH_2$ increase ($r = 0.98$ to $0.99$; $p < 0.05$, Fig. 3). The slope was steeper in diabetics than in non-diabetic

**Table 3.** Plasma insulin: basal values and increases over basal during arginine and arginine + glucose infusions in the control subjects with high and low insulin responses

| Groups | Basal values (mU/l) | Sum of increases over basal ($\mu U \cdot ml^{-1} \cdot 90\ min^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Arginine I | Arginine II | Arginine III | Arginine IV | Arginine III + glucose I | Arginine III + glucose II | Arginine III + glucose III |
| High responders | 24±1 | 53±12 | 102±13 | 236±30 | 255±29 | 392±54 | 540±95 | 1062±118 |
| Low responders | 24±1 | 52±24 | 70±12 | 106±29[a] | 125±29[a] | 246±67 | 333±50 | 445±60[a] |

Results are presented as in Table 2. [a] Statistical significance of differences between the two groups: $p < 0.001$

subjects, but was similar in high and low insulin responders.

Biphasic insulin responses were obtained for all doses of arginine both in high and low insulin responders. The increases in insulin and the $\alpha$-$NH_2$ were highly correlated in both groups ($r = 0.96$ and 0.97). The insulin response was lower in low responders than in high responders (Table 3). This difference was even more striking considering the higher blood glucose and $\alpha$-$NH_2$ levels in low insulin responders to arginine in all experiments.

Arginine infusions exerted modest non-significant effects on blood alanine concentration in the non-diabetic subjects. In contrast, in the insulin deficient patients IV arginine induced a decrease in alanine concentration (Fig. 4), which was time-related (Fig. 5) and dose-related with $\alpha$-$NH_2$ and IRG changes ($r = -0.97$ and $-0.98$ respectively).

### *Effects of Infusions of Glucose Together with Arginine*

As shown in Table 2, the increases in blood glucose were significantly higher in low than in high insulin responders, and considerably more so in the diabetics with fair metabolic control. In the diabetics with good control, the increases in blood glucose concentrations were similar to those of normal subjects (Table 2). The blood $\alpha$-$NH_2$ elevation following arginine was not influenced by the glucose or insulin infusions.

The effect of glucose III on the glucagon response to arginine III is shown in Figure 6. A significant decrease in plasma glucagon was obtained both in high and in low insulin responders. There was a linear relationship between the degree of inhibition of glucagon secretion and the log of the corresponding increase in blood glucose following the infusion of the three doses of glucose into the high and low insulin responders (Fig. 7). The degree of A cell inhibition by glucose was similar in these two groups but no inhibitory effect of glucose could be demonstrated in the diabetics with fair control (Figs. 6 and 7). However, when these patients were intensively treated with insulin, fasting plasma glucagon returned towards normal

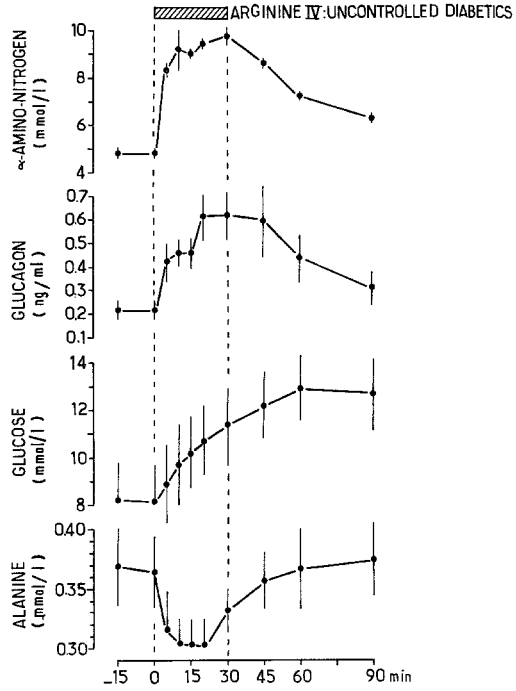


**Fig. 5.** Time course of $\alpha$-amino-nitrogen, glucagon, glucose and alanine during infusion of arginine IV in poorly controlled diabetics ($n = 10$). The hatched bar indicates the duration of infusion

(176±16 pg/ml) and the arginine infusion significantly suppressed the A cell response both with and without the administration of glucose (Figs. 7 and 8).

The addition of glucose to arginine significantly augmented the insulin responses in high as well as low insulin responders (Table 3).

### *Effect of Insulin-Induced Hypoglycaemia*

Insulin-induced hypoglycaemia to a mean value of 1.9±0.1 mmol/l in five aggressively treated diabetics, resulted in a plasma IRG of 220±33 pg/ml at 30 min. This value was similar to that obtained after an overnight fast in the 10 well-controlled patients (176±16 pg/ml).

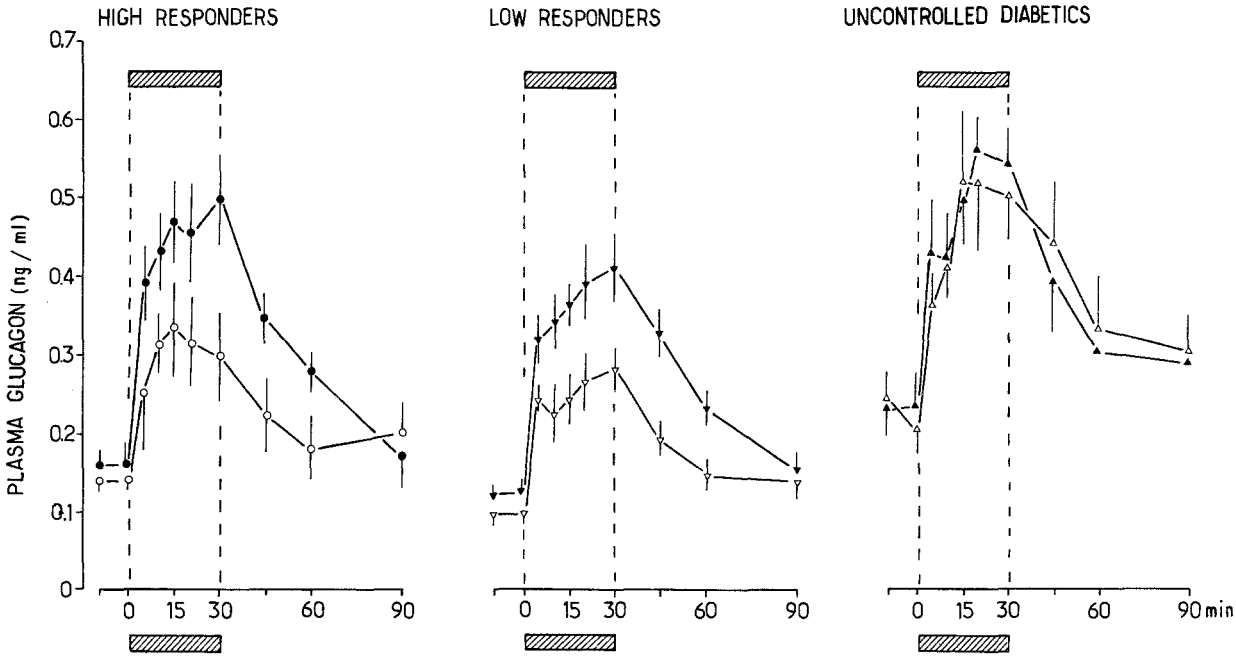


**Fig. 6.** Plasma glucagon response to arginine III (closed symbols) and arginine III + glucose III (open symbols) in high insulin responders (●, $n = 14$, ○, $n = 12$), low insulin responders (▼, $n = 10$, ▽, $n = 6$) and diabetics (▲, $n = 10$, △, $n = 10$) in absence of good metabolic control. Hatched bars indicate the duration of infusions

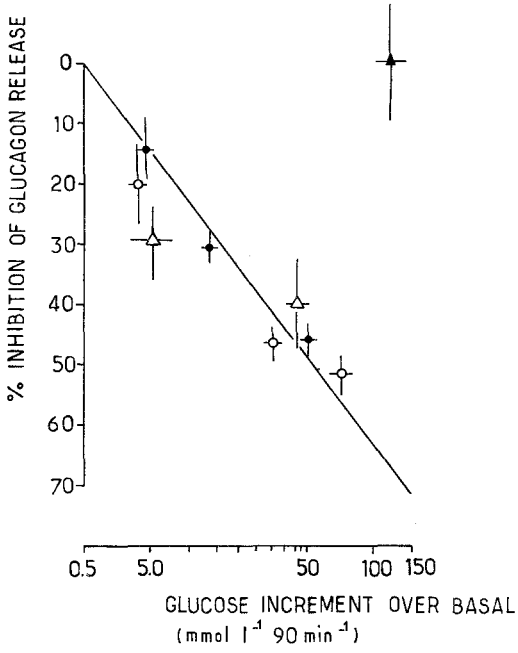


**Fig. 7.** Inhibition of the glucagon response to arginine III by glucose I, II and III. The glucagon response to arginine + glucose (sum of values over 90 min) has been calculated as percentage of the response to arginine alone, and plotted against the concomitant incremental blood glucose levels (sum of values over 90 min). High insulin responders (●, $n = 8$), low insulin responders (○, $n = 8$), uncontrolled diabetics (▲, $n = 10$) and tightly controlled diabetics (△, $n = 10$). Results are presented as mean ± SEM

## Discussion

Many of our results confirm findings that are already well established, such as islet A cell hyperactivity during poor control of blood glucose in diabetes [16–17], increased glucagon response to amino-acids [17–20] and non-suppressibility of glucagon release by glucose in diabetics [21]. In addition our dose-response studies suggest that in diabetics it is the capacity rather than the sensitivity of the A cell response to arginine which is augmented.

It is shown here clearly that A cell dysfunction in insulin dependent diabetics is related to metabolic changes and disappears with good control of the blood glucose. Several recent studies have also demonstrated that fasting glucagon levels, the glucagon response to arginine, and its suppressibility by glucose can be restored to normal or near-normal by intensive insulin therapy [21–27]. Not only insulin dependent patients but also insulin independent diabetics demonstrate hyperactivity of A cells [28]. In these patients only very high doses of insulin could normalize the glucagon levels [28], but such subjects display a relative insulin deficiency and insulin resistance.

Supraphysiological doses of insulin are usually necessary to achieve A cell normalization in diabetic man or animals. However, this may simply mean re-

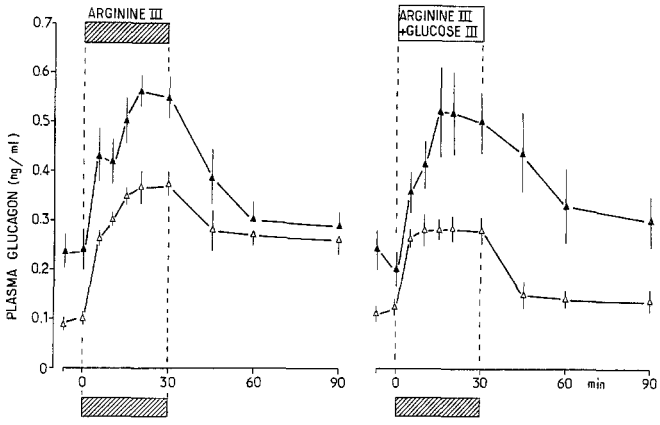


**Fig. 8.** Plasma glucagon responses to arginine III (left panel) and arginine III + glucose III (right panel) in diabetics in the absence (▲, $n = 10$) and in the presence (△, $n = 10$) of tight metabolic control. Results are presented as mean ± SEM

constitution of the high insulin concentrations which are supposed to exist at the vicinity of A cells [29]. When minute amounts of insulin were continuously infused into diabetics by a glucose controlled device or an open loop system [8, 27] several days or weeks were necessary to normalize the plasma glucagon level. This inability of an acute insulin infusion to correct A cell reaction to amino acids and glucose in diabetic patients [22–24] is not necessarily in conflict with our results since complete normalization of the metabolic state can hardly be achieved under short-term conditions.

Insulin reduces the A cell response in vitro to amino acids by the pancreas of alloxan-diabetic rats [32] and to a lesser extent by the pancreas from streptozotocin-diabetic rats [33]. This insulin sensitivity of diabetic A cells, albeit incomplete in some cases, is consistent with the suppressibility of normal A cells by glucose only in the presence of insulin.

Our observations in low insulin responders do not support the concept of a primary A cell abnormality in insulin independent diabetes. A markedly higher probability to develop diabetes has been established by the follow-up of these subjects over a 1–10 year period [10], which strongly suggests that this condition is somehow related to insulin independent diabetes. The low insulin responders displayed normal fasting glucagon levels and a normal A cell suppression of glucose. Their A cell responses to arginine were, if anything, slightly lower than normal, possibly because of the slightly higher blood glucose values following arginine infusion. Our findings are in agreement with other results in the literature. Thus, normal A cell response to arginine and to glucose was observed in non-diabetic Pima Indians with both parents diabetic [30, 31, 34], in the mothers of big neonates with gestational diabetes [35] and in a monozygotic triplet with two diabetic brothers, who was studied for 2 years before the appearance of diabetes [36]. These results are at variance with two other studies on the discordant monozygotic twins of diabetics [37] and first degree relatives of diabetics [38] where the insulin response to glucose was slightly impaired and the glucagon was not suppressed.

The finding of somewhat lower fasting and post-arginine glucagon levels in our low insulin responders is of interest. It was demonstrated [39] that these subjects have a lower than normal splanchnic glucose production rate, probably thus partially compensating for their insulin deficiency. Whether these two observations are causally interrelated cannot be decided from the present study.

Once diabetes is established glucagon oversecretion may play an important role in aggravating the characteristic metabolic disturbances [40–48] but our results support the idea that A cell dysfunction is not a primary cause of the disease.

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