Glucagon Responses to Arginine in Chronic Pancreatitis

Possible Pathogenic Significance in Diabetes

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SUMMARY

The effect of arginine infusion on pancreatic glucagon, insulin and glucose was studied in fourteen patients with chronic 'alcoholic' pancreatitis and seven control patients. There appeared to be two types of pancreatitis, one with concomitant decrease in glucagon and insulin secretion and the other with severe insulinopenia and relative hyperglucagonemia. The bihormonal derangement mimics abnormalities that occur with genetic diabetes. The relative hypersecretion of glucagon in chronic pancreatitis may be a consequence of insulinopenia rather than a primary abnormality. DIABETES *23:* **257-63, April, 1974.**

Recent studies suggest that, in genetic diabetes, circulating glucagon levels are inappropriately elevated for the raised glucose concentrations and that hyperglycemia does not suppress arginine-stimulated hyperglucagonemia.[3,6,8] Since hyperglycemia regularly suppresses glucagon release, provided insulin secretion is intact,[2,3] it is not clear whether hyperglucagonemia with genetic diabetes is a causal factor or consequent upon insulinopenia.[1,6] The effect of insulin deficiency on glucagon secretion can be studied in a simpler context, in chronic pancreatitis, than in genetic diabetes.

We present data on pancreatic and gut glucagon and insulin responses to arginine infusion in patients with pancreatitis that demonstrate a greater functional impairment of the beta cells than of the alpha cells. We further suggest that the relative hypersecretion of glucagon in some patients may be a consequence of insulinopenia and, therefore, mimics the abnormality in 'genetic' diabetes.

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PATIENTS AND METHODS

Fourteen patients who had had one or more attacks of pancreatitis associated with long-standing overindulgence in alcohol were studied. The diagnosis of pancreatitis was confirmed by radiologic demonstration of pancreatic calcification in four, and by laparotomy in five. The remaining five patients had histories typical of alcohol-induced pancreatitis[12] and grossly abnormal pancreatic bicarbonate and enzyme responses after secretin and pancreozymin stimulation,[13] i.e. a mean bicarbonate concentration < 60 mEq./L. and amylase < 5 U./kg. of Boots secretin and pancreozymin, respectively. Three of these five patients had had clinical and radiologic signs of pancreatic cysts in the past. The subjects included eleven males and three females twenty-eight to sixty-one years old. None was taking or had taken antidiabetic agents at the time of the examination. There was no evidence of malabsorption syndromes, none was grossly obese, and none had a family history of diabetes.

Of the seven control subjects studied—all males twenty-six to thirty-seven years old—five had never consumed alcohol and two were 'social drinkers'. None had any manifestations of pancreatic disease, and none was grossly obese or had a family history of diabetes.

Arginine infusion tests were performed after an overnight fast. A polythene canula was inserted into a vein in the antecubital fossa, a specimen of blood was taken, and 30 gm. of L-arginine HCl in 150 ml. of isotonic saline was then infused over thirty minutes. Blood was taken for measurement of glucose, immunoreactive insulin (IRI), pancreatic glucagon-like immunoreactivity (PGLI) and gut glucagon-like im-

munoreactivity (GGLI) at -30, -15, 0, 5, 10, 20, 30, 35, 40, 50 and 60 minutes. Blood for glucagon assay was mixed with heparin and aprotinin [Trasylol (Bayer)] and centrifuged, and the separated plasma was immediately deep frozen until assayed by modifications of the radioimmunoassay procedure of Hazzard et al.[14] Plasma was extracted by the method of Heding[15] and 'free' from 'bound' hormone was separated by a dextran-charcoal method.[16] Two glucagon antibodies were used during the studies: YY 89 (final titer 1:6,750), which was PGLI-specific, and YY91 (final titer 1:4,500), which showed 10 per cent cross-reactivity with gut extract. For total glucagon-like immunoreactivity (GLI), strongly cross-reacting antisera, YY57 and YY71, were used and gut glucagon-like immunoreactivity (GGLI) was estimated by subtracting PGLI from total GLI.

Blood for IRI was allowed to stand and the separated serum was deep frozen until assayed, using Amersham insulin kits. Glucose was measured on the Technicon AutoAnalyzer by the ferricyanide method of Hoffman.[17]

The mean fasting values for glucose IRI and GGLI and PGLI were the means of the -30, -15 and 0 minute values for each except that if one value was obviously different it was omitted. The increments of IRI and PGLI for each of the eight samples taken during the sixty minutes of the test were added to give the total IRI or PGLI increments. When, for technical reasons, one sample was not assayed in a subject, the IRI or PGLI level for that time was determined by extrapolation to the line joining the levels at the time periods immediately before and after the missing value.

On the basis of the insulin responses to arginine, the patients were divided into two groups: Group I patients had 'normal' insulin responses (39 to 337.5 μU./1 ml./1 hr.); Group II patients were insulinopenic (0 to 37 μU./1 ml./1 hr.).

All subjects were also examined by 50 gm. oral glucose tolerance tests. Results were considered indicative of diabetes if two of three glucose values —fasting, 60 minute or 120 minute—were above 120, 180, and 140 mg./100 ml., respectively.

The results were analyzed statistically using Student's *t* test for significance of differences between means.

RESULTS

Control subjects had normal fasting plasma glucose levels and normal or slightly elevated (one subject) fasting serum IRI levels. Mean fasting plasma PGLI was 120.6 ± 24.2 $\mu\mu$g./1 ml. (range 40 to 192 $\mu\mu$g./1 ml.) and mean fasting GGLI 42.6 ± 9.6 $\mu\mu$g./1 ml. (range 10 to 90 $\mu\mu$g./1 ml.) (table 1). The peak plasma glucose levels occurred during or five minutes after the end of the arginine infusion. There was a mean rise of 14.6 mg./100 ml. above the mean fasting level, followed by a mean fall of 22.7 mg./100 ml. to a 'trough' which occurred ten to thirty minutes after the end of the infusion (table 2 and figure 1).

IRI and PGLI began to rise within five minutes after the start of the infusion, and the mean peak occurred at thirty minutes (figure 2). Five of the seven controls showed a significant increase in plasma gut GLI in response to arginine (table 2), the mean maximal increment for the group being 61.7 ± 22.4 $\mu\mu$g./1 ml. Peak levels occurred five to thirty-five minutes after the start of the infusion and then tended to fall toward basal levels. Levels fluctuated considerably in each individual throughout the test.

Group I patients had fasting values of glucose, IRI, PGLI and GGLI not significantly different from control values (table 1). The glucose response to the arginine infusion was similar to the reaction in the control group, showing a mean rise of 10.4 mg./100 ml. from fasting to peak, and a subsequent mean fall of 18.1 mg./100 ml. (figure 1). The mean total IRI increment of 100.6 ± 35.5 μU./1 ml./1 hr. was not significantly different from that in controls; the range was similar, 39 to 337.5 μU./1 ml./1 hr., but five of the eight patients had a 'low normal' response (figure 3). The mean maximum and total PGLI increments were significantly lower than in the controls (171.3 ± 16.2 $\mu\mu$g./ml. ($p < 0.01$) and 428.0 ± 93.8 $\mu\mu$g./1 ml./1 hr., respectively ($p < 0.01$) (table 2). The mean fasting GGLI level was 30.8 ± 10.5 $\mu\mu$g./1 ml. Six of these eight patients had a significant rise in GGLI levels above fasting with a mean maximum increment of 75.4 ± 19.9 $\mu\mu$g./ml., not significantly different from findings among controls (tables 1 and 2). Two in this group had glucose tolerance tests indicative of diabetes.

Group II patients (insulinopenic) had a higher mean fasting blood glucose level (138 ± 26 mg./100 ml.) than both the control ($p < 0.05$) and the group I subjects ($0.05 < p < 0.1$). However, the range was much greater, 87 to 285 mg./100 ml., and in three patients the level was below 100 mg./100 ml. Fasting IRI, PGLI and GGLI levels were not significantly different from the values in the other two groups: 18.9 ± 2.7 μU./ml., 72.8 ± 32.6 $\mu\mu$g./ml. and 43.8

TABLE 1

Comparisons of fasting levels of glucose, IRI, PGLI and GGLI in control subjects and patient groups

Patients	Glucose (mg./100 ml.)	IRI (μU./ml.)	PGLI (μμg./ml.)	GGLI (μμg./ml.)
Group I ('Normal' insulin responses)				
BT	97	21.5	230	70
JB	94	20.3	80	10
JM	71	21.7	30	43.3
MI	116 (D)*	17.5	40	10
JE	92	14.5	106	10
JP	98	8	160	13.3
JM	124 (D)	18	54	10
TD	82	18	150	80
Mean ± S.E.M.	97 ± 6.0	17.4 ± 1.6	106.3 ± 24.5	30.8 ± 10.5
Group II (Insulinopenic)				
Patients				
LH	155 (D)	13	20	10
WH	139 (D)	24	40	43.3
HR	87 (D)	21.5	110	13.3
JS	92	10.6	220	66.6
JF	99	17	20	73.3
LM	258 (D)	27.6	26.6	73.3
Mean ± S.E.M..	138 ± 26.4	18.9 ± 2.7	72.8 ± 32.6	46.6 ± 11.9
Control Group (7)				
Mean values:	92.1 ± 1.3	18.7 ± 3.5	120.6 ± 24.2	42.6 ± 9.6

The mean fasting glucose level in group II patients was significantly higher than in the controls ($p<0.05$). However, mean fasting glucose levels in groups I and II and the mean fasting IRI, PGLI and GGLI levels in controls and both patient groups were not significantly different.

*(D) Glucose tolerance test suggestive of diabetes.

± 10.5 μμg./ml. respectively (table 2). However, this group was characterized by very low or absent insulin responses and a high rise of glucose during the arginine infusion test; the glucose level decreased only slightly immediately after the peak (figure 1). In only one patient was the glucose response normal, despite a low total IRI increment. The mean total IRI increment was 16.4 ± 6.6 μU./1 ml./1 hr. (0 to 37 μU./1 ml./1 hr.), which is significantly lower than in the control subjects ($p < 0.025$) and group I patients ($p < 0.05$). The PGLI responses in both patient groups appeared to be delayed (figure 2); however, mean values at individual times did not differ significantly. The mean maximal PGLI increment was 350.7 ± 70.3 μμg./1 ml., similar to the control value but significantly higher than the mean value for group I ($p< 0.01$). Similarly, the mean total PGLI increment was equivalent to that of the controls, 1,017.3 ± 192.5 μμg./1 ml./1 hr., but much higher than the mean value in group I (428.0 ± 93.8 μμg./1 ml./1 hr., $p < 0.01$, table 2, figure 3). The mean glucose rise was 40.0 ± 7.9 mg./100 ml. which was significantly greater than the 14.5 mg./100 ml. among the controls ($p < 0.025$) and the 10.4 mg./100 ml. for group I patients ($p < 0.0025$) (figure 1). Three of these six patients did not have a significant rise in GGLI above fasting levels; the mean maximum GGLI increment was 46.7 ± 33.5 μμg./1 ml., which is not significantly different from values for the controls and the group I patients (tables 1 and 2). In this group of six patients, the glucose tolerance test was suggestive of diabetes in four, including three with fasting hyperglycemia and one with fasting euglycemia; two reacted normally to glucose tolerance tests.

DISCUSSION

In the control group, fasting glucose, insulin and PGLI levels and responses to infused arginine were within the range previously designated normal.[6,8] In six of the seven subjects the pattern of the PGLI response was similar to that reported in other series, and the maximum increment was at least 180 μμg./ml.[8] The seventh subject had normal glucose and IRI responses, but the PGLI showed no rise, for which we have no ready explanation.

The group I patients, with 'normal' insulin secretion, and the group II (insulinopenic) patients could

TABLE 2

Total IRI increments, glucose changes and total and maximal PGLI increments during arginine infusion test in patients with pancreatitis and controls

Group I Patients	Total IRI increment (μU./ml./hr.)	Glucose rise (mg./100 ml.)	Glucose fall (mg./100 ml.)	Total PGLI increment (μμg./ml./hr.)	Maximum PGLI increment (μμg./ml.)	Maximum GGLI increment (μμg./ml.)
BT	337.5	19	−31	490	210	70
JB	127.6	12	−16	960	200	60
JM	84.4	16	−16	498	170	−43.3
MI (D)*	79.0	19	−16	120	120	90
JE	50	5	−19	520	154	100
JP	44	2	− 8	420	160	126.7
JM (D)	43 (40 min.)	3	−13	226 (30 min.)	246	70
TD	39	7	−26	190 (50 min.)	110	60
Mean ± S.E.M.	100.6 ± 35.5	10.4 ± 2.5	−18.1 ± 2.6	428.0 ± 93.8	171.3 ± 16.2	75.4 ± 19.9
Group II						
Patients						
LH (D)	37	23	−16	1,088	280	70
WH (D)	29	30	−11	380	140	26.7
HR (D)	26	18	−25	1,140	350	56.7
JS	6.2	65	+35	530	660	−6.6
JF	0	50	−16	1,400	300	−53.3
LM (D)	0	56	− 2	1,566	374	186.7
Mean ± S.E.M.	16.4 ± 6.6	40.3 ± 7.9	−5.8 ± 8.7	1,017.3 ± 192.5	350.7 ± 70.3	46.7 ± 33.5
Control subjects (7)						
Mean values	186.4 ± 69.2	14.6 ± 2.8	−22.7 ± 1.8	1,226.3 ± 373.3 (6)	279.3 ± 40.1 (6)	61.7 ± 22.4

*(D) Glucose tolerance test suggestive of diabetes.

Mean total IRI increments of group I patients who had 'normal' insulin responses and control subjects were not significantly different, but both were significantly ($p < 0.025$) greater than the mean increment in group II (insulinopenic) patients.

The mean glucose rise and fall during arginine infusion tests were similar in controls and group I patients, while the mean glucose rise in group II subjects was significantly ($p < 0.005$) greater and the mean fall was less ($p < 0.05$).

The high rise in glucose in group II patients was associated with significantly greater maximal ($p < 0.025$) and total PGLI increments ($p < 0.01$) than in the group I patients, but the PGLI levels were not significantly different from levels in the control subjects.

Fasting levels and maximum increments of GGLI in controls and patient groups were not significantly different. In three patients GGLI levels fell during the test.

not be differentiated on the basis of age, number of attacks of pancreatitis, alcoholic intake, presence of pancreatic calcification or hepatomegaly, or the degree of exocrine pancreatic insufficiency as measured by the secretin/pancreozymin tests. Neither the fasting blood glucose level nor the response to a 50 gm. oral glucose load was of value in predicting the PGLI response to arginine, although obviously, the grossly insulinopenic patients in group II would be more likely to be diabetic by glucose tolerance criteria. Nevertheless, three patients in this group had fasting normoglycemia and two had normal glucose tolerance, while one diabetic patient in group I had fasting hyperglycemia and one had 'chemical diabetes'.

Mean fasting GGLI levels in the controls and patient groups were not significantly different. Only five of the seven controls showed an increase in GGLI above mean fasting levels, and peak levels occurred at any time during or after the infusion. In any individual the GGLI peak was not related to peak IRI levels, and a relationship, if any, is unclear. Nine of the fourteen patients had a significant rise in GGLI in response to arginine; maximum GGLI increments were of similar magnitude to those in controls, and no relationship between peak GGLI and IRI levels could be demonstrated. The response of GGLI to arginine infusion differs from that reported earlier, which indicated pancreatic specificity of the stimulus, but may relate to our method of estimation and reflect interference by pancreatic glucagon in the assay.

The patients indeed comprised two fairly distinct groups. Group I showed normal glucose and IRI responses, but the mean PGLI responses to arginine, although within the reported normal range,[6,8] were significantly lower than in our controls. In most patients in this category there were 'low normal' IRI responses and generally low PGLI responses, both attributable to islet damage.[19,20] It is assumed that the

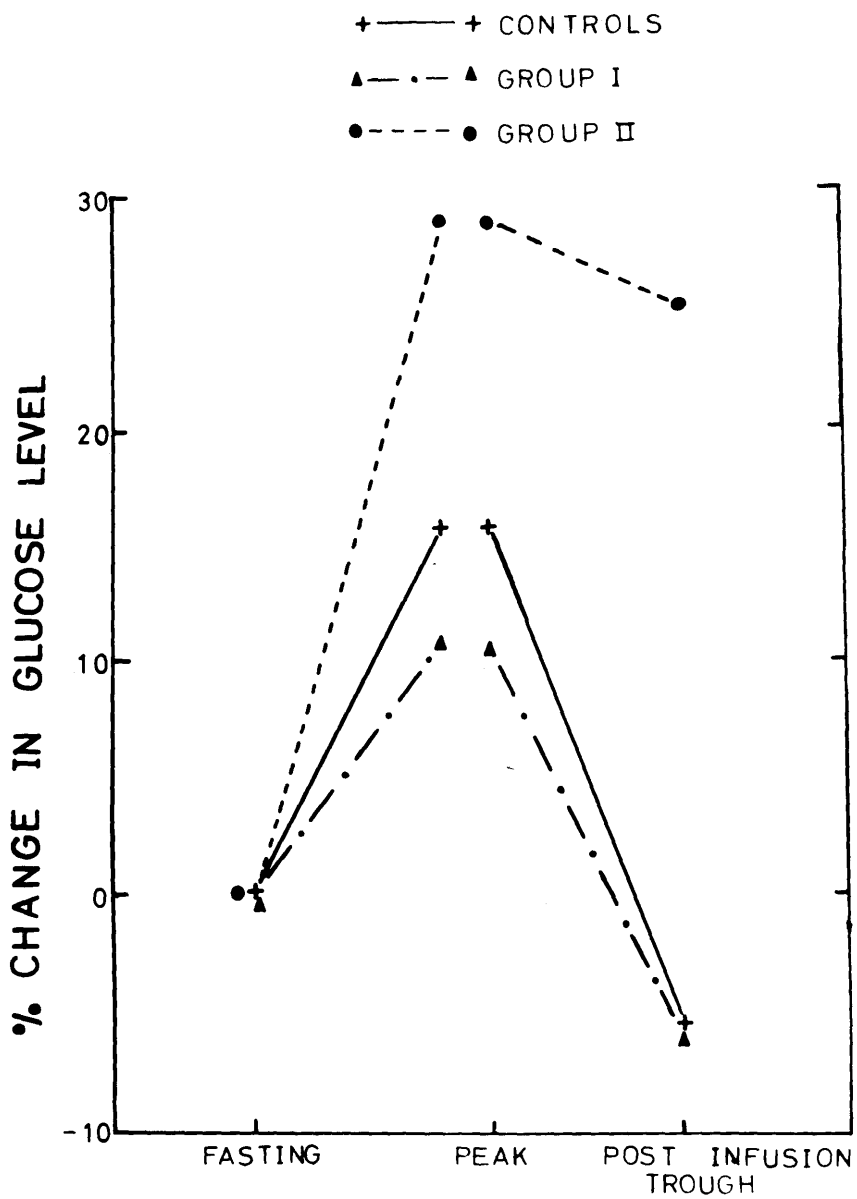

FIGURE 1

The glucose rise and fall in response to arginine in controls, insulin secretors (Group I) and insulinopenic (Group II) patients.

normal glucose responses and absence of hyperglycemia were due to adequate IRI responses in the presence of relatively low PGLI.[1]

In group II patients the responses to arginine infusion were similar to those described in genetic diabetes;[6,8] there was a wide range of fasting glucose concentrations in the presence of normal fasting IRI and PGLI levels. The mean rise of glucose was significantly greater than in the controls and group I patients and was of the same magnitude as seen in 'genetic' diabetes.[6,8] The increased glucose response was associated with a low or absent IRI response, but with a *normal* mean PGLI response, which was significantly greater than in group I.

The mean maximal and total PGLI increments in response to arginine in group II (insulinopenic patients) were significantly greater than in group I, despite fasting hyperglycemia and/or the high rise in glucose levels, both of which would be expected to reduce or prevent glucagon secretion.[2-6]

In man,[7] as in animals,[21] the suppressive effects of high glucose concentrations on glucagon secretion do not occur in the absence of insulin. Thus, severe insulin deficiency per se may explain the inappropriately

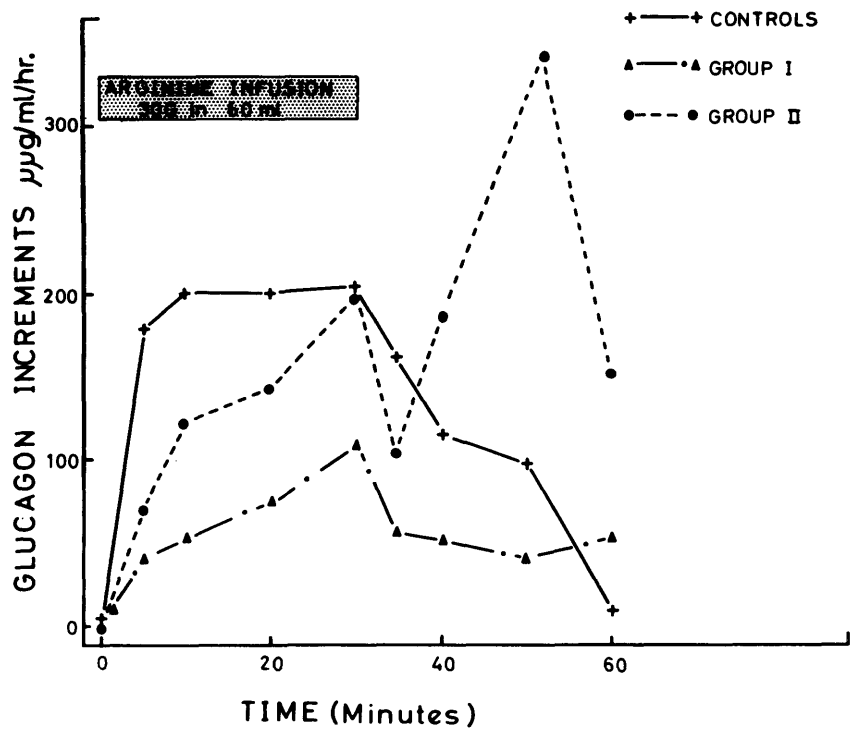

FIGURE 2

PGLI increments above basal concentrations in controls, Group I ('normal' insulin responses) and Group II (insulinopenic). Individual values do not differ significantly.

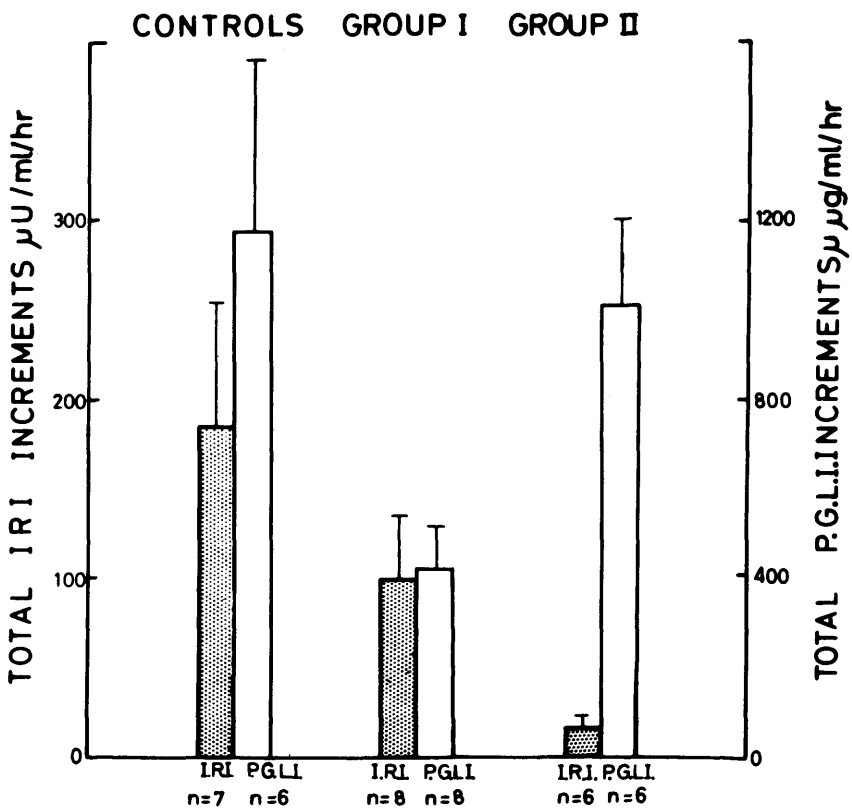

FIGURE 3

Total IRI and PGLI increments above basal in controls and patient groups. There is a significant parallel decrease in IRI and PGLI responses to arginine in Group I, while insulinopenic patients in Group II have 'normal' glucagon responses.

high PGLI increments in the presence of the arginine-induced hyperglycemia observed in our insulinopenic patients. The tendency to relative hyperglucagonemia in genetic diabetes[1,6] may have no pathogenic significance, since insulinopenia and relative hyperglucagonemia accompany acquired diabetes of chronic pancreatitis.

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