Suppressible Glucagon Secretion in Young, Ketosis-Resistant, Type "J" Diabetic Patients in India

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SUMMARY

Plasma glucagon levels were measured in young individuals with severe, insulin-dependent, juvenile-onset diabetes mellitus to study whether differences in glucagon secretion were related to ketosis proneness and resistance. Fasting glucagon levels were similarly elevated in both classical, ketosis-prone, type I diabetic subjects and ketosis-resistant, type "J" subjects [70 ± 7 pmol/L (mean ± SEM) and 81 ± 10 pmol/L, respectively] compared with nonobese, nondiabetic controls (36 ± 3 pmol/L, $P < 0.01$). After oral glucose administration, however, glucagon responses were strikingly dissimilar in the two groups. In type I diabetic individuals, glucagon rose paradoxically during OGTT, by 21 ± 4 pmol/L, an increase of 33 ± 10%; on the other hand, glucagon levels in type "J" diabetic individuals fell by 28 ± 7 pmol/L, a decrease of 33 ± 5%. There was no measurable increase in plasma free insulin during OGTT in either group. Postprandial glucagon suppressibility may be relevant to the ketosis resistance that is characteristic of type "J" diabetes. DIABETES *32*:1168–1171, December 1983.

Young, lean, insulin-dependent diabetic individuals from India, with an atypical absence of ketosis after insulin withdrawal, have been described.[1–3] They are similar to the growth-onset diabetic patients first described by De Zoysa,[4] and termed type "J" diabetic individuals by Hugh-Jones.[5] These individuals have a distinctive clinical and biochemical profile.[6,7] Severe diabetes of sudden onset requiring large amounts of insulin for control is characteristic, but insulin withdrawal is not followed by ketosis. The reason for the remarkable absence of ketosis has not been completely elucidated. In the present study, plasma insulin and glucagon levels were estimated in young ketosis-prone and ketosis-resistant diabetics, in view of the postulated role of glucagon in ketogenesis.

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SUBJECTS

Type "J" diabetic subjects were 12 diabetic individuals, 13–25 yr old, with a history of having stopped insulin treatment (generally due to poverty) for at least 1 mo before admission. There was no rise in plasma acetone levels during a 10-day observation period without insulin.

Type I diabetic subjects included six patients, 11–20 yr of age, with at least one documented episode of ketoacidosis and a rise in plasma acetone concentration after insulin withdrawal under strict observation in hospital.

Controls were 12 healthy, apparently normal individuals, 20–38 yr of age, with no clinical or biochemical evidence of diabetes and with no family history of diabetes.

METHODS

All the diabetic subjects were admitted to the hospital and treated with multiple injections of soluble insulin until 24-h urine was totally free of glucose, and normoglycemia was achieved. An oral glucose tolerance test was then performed with 1.75 g glucose/kg body wt, about 15 h after the last meal and accompanying injection of short-acting insulin.

Venous blood was collected in prechilled glass tubes containing 1.5 mg disodium EDTA per ml blood. Benzamidine, a proteolytic inhibitor, was added to a final concentration of 0.03 M. Plasma was separated and stored at −20°C until assay. Blood was collected in sodium fluoride for glucose estimation.

Analytic techniques. Blood glucose was estimated by the Nelson-Somogyi method[8] and plasma free fatty acids (FFA) by the method of Laurell and Tibbling,[9] using the color reagent of Itaya.[10] Plasma free insulin was assayed by a double antibody radioimmunoassay[11] after removal of endogenous insulin antibodies with polyethylene glycol.[12] Insulin antibody

TABLE 1
Glucose tolerance data in different clinical types of young Indian diabetic subjects (mean ± SEM)

	Controls (N = 12)	Type I (N = 6)	Type "J" (N = 12)
Fasting blood glucose (mmol/L)	4.63 ± 0.14	13.0 ± 1.6*	14.9 ± 1.2*
Peak blood glucose (mmol/L)	7.5 ± 0.24	27.4 ± 2.1*	29.2 ± 2.2*
Total glucose area (mmol·L^{-1}·h)	14.7 ± 0.3	54.4 ± 5.1*	59.2 ± 4.2*
Fasting plasma free insulin (mU/L)	17.1 ± 1.2	5.7 ± 1.2*	7.9 ± 1.4*
Total insulin area (mU·L^{-1}·h)	165 ± 3.8	23.3 ± 2.9*	26.1 ± 4.6*
Insulin requirements (U/day)	—	54 ± 6†	125 ± 7†

Difference between diabetics and controls tested by one-way analysis of variance and multiple range test: significant at *$P < 0.01$. †$P < 0.001$ tested by *t* test.

was purchased from Wellcome Research Labs., Beckenham, England (lot K 4671). Glucagon was assayed by the method of Heding,[13] except that bound and free hormone were separated by using guinea pig anti-rabbit globulin serum. Antiglucagon serum 30K was obtained from Dr. Roger H. Unger, Dallas, Texas. ^{125}I-labeled insulin and glucagon were prepared by using chloramine-T.[14,15] Minimum detectability limits (95% level) in the radioimmunoassays were 4.0 mU/L for insulin and 25 pmol/L for glucagon. Insulin antibodies were assessed by the method of Kansal and Boshell,[16] except that the separation step was carried out with polyethylene glycol.

Statistical analysis. Results were analyzed by one-way analysis of variance, and the means were compared by Neuman-Keuls multiple range test for unequal sample size.[17] Plasma glucagon and FFA levels were compared using Duncan's range test after ANOVA.[18] Areas under the glucose and insulin curves were measured by planimetry.

RESULTS

Nutritional status. Both groups of diabetic subjects were poorly nourished. Body-build indices (wt/ht^2 × 100) were lower ($P < 0.001$) in both type I (0.14 ± 0.01, mean ± SEM) and type "J" diabetic patients (0.16 ± 0.01) than in the controls (0.22 ± 0.02). Skinfold thickness was also less in both diabetic groups (type I: 6.5 ± 0.4 mm; type "J": 8.9 ± 1.1 mm) than in the controls (12.6 ± 0.5, $P < 0.01$).

Glucose tolerance (Table 1). All the patients had moderate-to-severe diabetes with blood glucoses greater than 10 mmol/L (fasting) and 20 mmol/L (peak).

Plasma free insulin (Table 1, Figure 1). The mean fasting plasma free insulin levels in both groups of diabetic patients were lower than in the controls. The response of insulin to oral glucose was negligible in both groups, with no significant rise in free insulin levels at any stage during the OGTT.

Fasting plasma FFA was elevated in both type I (674 ± 54 μmol/L) and type "J" diabetic subjects (636 ± 39 μmol/L) compared with the normal subjects (492 ± 79 μmol/L, $P < 0.05$).

Plasma glucagon levels (Table 2, Figures 2 and 3). The fasting glucagon levels of both type I and type "J" diabetic subjects were twice as high as the levels seen in the control subjects ($P < 0.01$). The glucagon response to the oral glucose load was, however, strikingly different in the two groups of diabetic individuals. While type I subjects showed a paradoxical rise in plasma glucagon during the OGTT, levels in the type "J" individuals were suppressed (Figure 2). Although the actual magnitude of the fall in glucagon was greater than in controls when corrected for initial value by covariate analysis or by expressing it as percentage change from fasting, the responses were not significantly different (Figure 3).

Insulin requirements and insulin antibodies (Table 1). Type I diabetic patients required 36–80 U of soluble insulin in multiple injections for adequate glycemic control. Insulin requirements in the type "J" subjects, however, ranged from 90 to 170 U per day. In none of these patients was the insulin binding to plasma found to exceed 35%; in fact, in 7 of the 12, binding did not exceed 10%.

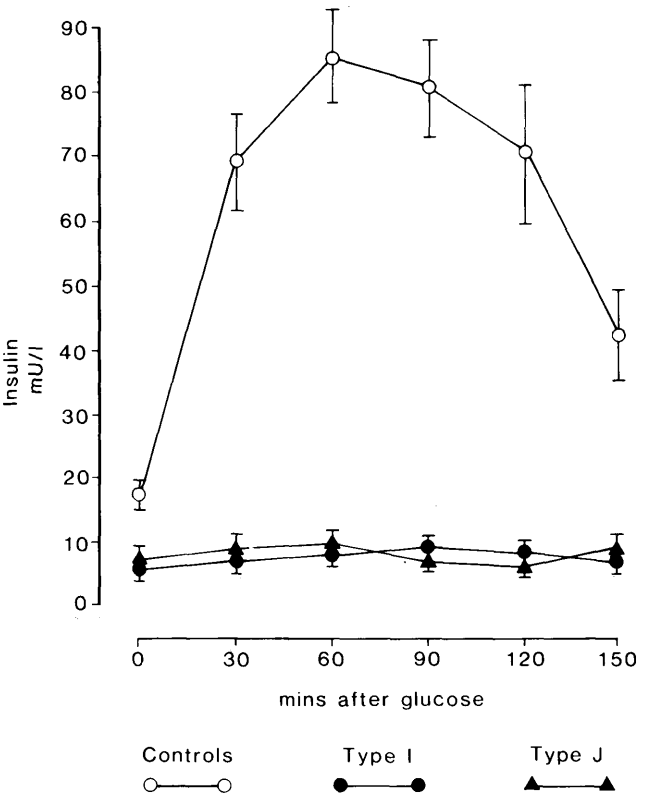

FIGURE 1. Plasma free insulin levels (mean ± SEM) after an oral glucose load in young Indian diabetic subjects.

TABLE 2
Changes in plasma glucagon levels during OGTT in young Indian diabetics (mean ± SEM)

	Plasma glucagon (pmol/L)							
	Minutes after glucose load						Maximum change during OGTT	
Group	0	30	60	90	120	150	Δ Concentration	% Change
Controls (6)	36 ± 3	32 ± 2	28 ± 2	30 ± 1	31 ± 1	32 ± 2	−9 ± 2	−24 ± 3
Type I (6)	70 ± 7*	85 ± 7*	89 ± 9*	86 ± 8*	78 ± 8*	73 ± 6*	+21 ± 4†	+33 ± 10†
Type "J" (6)	81 ± 10*	68 ± 8*	62 ± 7*	62 ± 10*	64 ± 7*	71 ± 9*	−28 ± 7†	−33 ± 5

Figures in parentheses indicate the number of subjects in the group. Statistical significance, diabetics versus controls: *$P < 0.01$, Duncan's range test, after ANOVA; †$P < 0.001$, t test.

DISCUSSION

The present study was aimed primarily at examining the role of glucagon in the ketosis resistance of type "J" diabetic subjects seen in India. Hyperglucagonemia supervenes rapidly after insulin withdrawal in an insulin-deficient, growth-onset diabetic.[19-21] Furthermore, glucose loading does not suppress glucagon in diabetic individuals with the degree of hyperglycemia seen in the present study.[22-25] In type I diabetic patients, in fact, a paradoxical rise in glucagon occurs in response to a glucose load.[22,26,27] In the present study, type "J" diabetic subjects had elevated fasting glucagon levels similar to those seen in the type I subjects, after 15 h of fasting and withholding insulin. However, the glucagon responses to oral glucose were strikingly different: type I subjects showed a paradoxical rise, but type "J" individuals showed a suppression of glucagon. Although the actual magnitude of the fall was greater in the type "J" diabetic subjects than in the controls, it was not significantly different from the normal when it was corrected for initial value either by covariate analysis or by expressing it as a percentage change over the fasting level (Figure 3).

The mechanism by which glucagon suppression is brought about is not clear. It is possible that insulin may be secreted in amounts that escape detection peripherally, but are sufficient to exert a within-islet effect on the alpha-cell. Whatever the explanation, the phenomenon is extremely unusual in diabetic patients, though not in itself unique. It has been reported in mildly diabetic individuals, but a significant, though delayed and subnormal, insulin response was seen in these patients.[28,29] On the other hand, the degree of hyperglycemia seen in our type "J" diabetic subjects is characteristic of fairly severe diabetes, and reflects reasonably

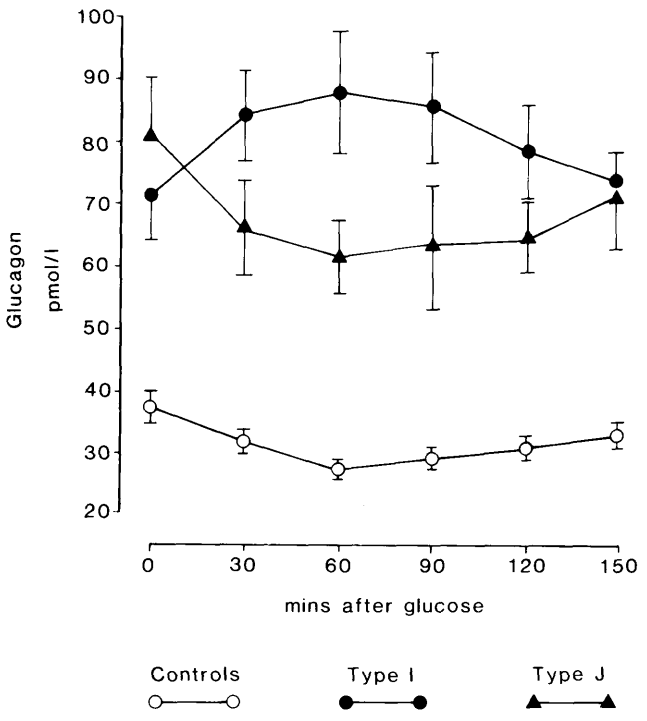

FIGURE 2. Plasma glucagon responses to oral glucose loading in young Indian diabetic subjects (mean ± SEM).

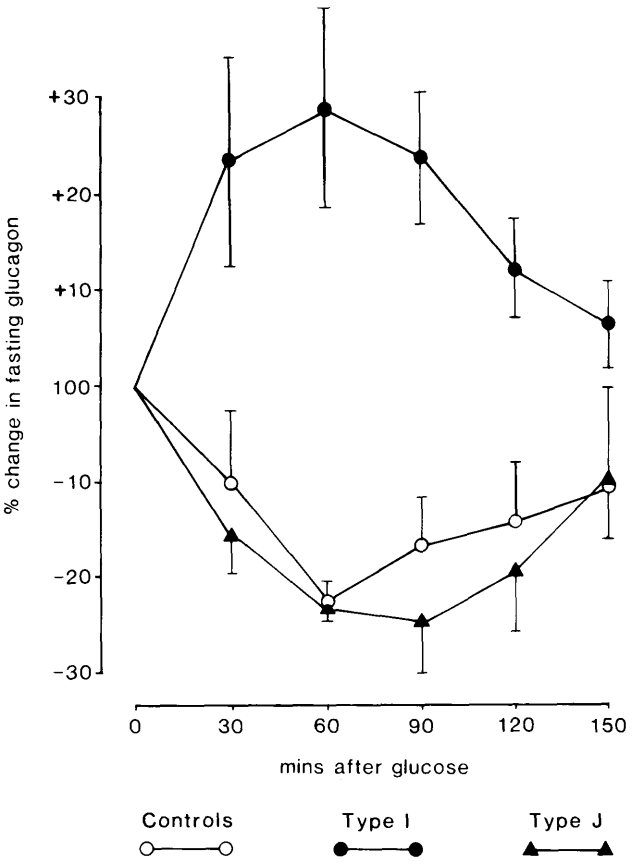

FIGURE 3. Change in glucagon during OGTT expressed as a percentage of the fasting glucagon level (mean ± SEM).

advanced beta-cell failure. The absence of a detectable rise in insulin after glucose supports this contention, although the diagnosis of total beta-cell failure would require C-peptide measurements. Such documentation would be essential before ketosis resistance in type "J" diabetic individuals could be attributed to postprandial glucagon suppressibility, because only small amounts of insulin, secreted into the portal vein and not detected peripherally, may be enough to inhibit ketogenesis in the liver.[30] Even smaller amounts, not even detectable with C-peptide measurements, could probably mediate the within-islet effect on the alpha-cell referred to earlier.

The pathophysiologic basis for ketosis resistance in type "J" diabetic subjects is still conjectural. Inadequate availability of substrate (FFA) has been thought to be important,[31] but our own observations suggest that this may not be so, because FFA was equally raised in both groups after 15 h of fasting and withholding insulin. Another possibility is that ketosis resistance or proneness may be influenced, at least in part, by nutritional factors. Undernourished subjects have low levels of plasma carnitine that rise subnormally after oral loading with lysine, the precursor of carnitine; these abnormalities are corrected by dietary lysine supplementation.[32-34] Elevated hepatic carnitine is a key factor in ketogenesis,[35] and undernourished type "J" diabetic patients may have abnormalities in carnitine metabolism that contribute to ketosis resistance. Finally, ketosis-resistant, type "J" diabetes may be nothing other than a nutritionally modified form of maturity-onset diabetes of youth (MODY). This seems unlikely in view of the marked difference between the two syndromes: MODY is characterized by asymptomatic or mild diabetes that does not progress and responds to diet and sulfonylureas,[36] whereas type "J" diabetes is a syndrome of severe hyperglycemia, acute onset, and relative insulin resistance. The absence of a family history is another important difference.

The syndrome of type "J" diabetes has not, to our knowledge, been reported outside the tropics. Its differences from the classical diabetic syndromes may thus be environmentally mediated. Of these, ketosis resistance is the dominant feature. The role of postprandial glucagon suppression in the pathophysiology of this syndrome is uncertain. It is, however, another atypical feature that serves to highlight the distinctive character of type "J" diabetes, with its unusual clinical and biochemical picture.

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