Juvenile Diabetes Mellitus, a Deficiency in Insulin

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SUMMARY

The plasma insulin, growth hormone, nonesterified fatty acids and glucose responses to the oral ingestion of glucose, the intravenous administration of tolbutamide and the infusion of arginine were studied in fourteen newly diagnosed juvenile diabetic children. Fasting plasma insulin levels did not differ significantly between diabetic and normal children, although the mean fasting blood glucose level of the diabetic children was threefold greater than that of the normal subjects No detectable plasma insulin response was observed in the diabetic subjects during all three tests. Fasting plasma growth hormone levels and plasma growth hormone responses were similar in both normal and diabetic children. The fasting free fatty acid level of the diabetic children was significantly higher than in normal children, but fell during all three tests. With use of a variety of sensitive procedures, no antibodies to human insulin were demonstrable in the sera of newly diagnosed juvenile diabetics. DIABETES *17*:27-32, January, 1968.

Juvenile diabetes mellitus is characterized by an abrupt onset, ketoacidosis and a poor response to oral hypoglycemic agents. It had been suggested originally on a clinical basis that this disease is associated with an absolute insulin deficiency. As early as 1951, Bornstein and Lawrence,[1] using an in vivo bioassay technic, reported the absence of insulin-like activity in the sera from two young diabetic patients. More recent support has been provided by investigators utilizing the sensitive and specific radioimmunoassay for plasma insulin. Berson and Yalow[2] and Ehrlich and Bambers[3] have reported low fasting values and little if any rise in serum immunoreactive insulin in response to glucose in a limited number of juvenile patients. Pathologic examination of the pancreases of young patients who died during the initial episode of ketoacidosis has revealed shrunken islets characterized by a marked deficiency of the beta cells and lymphocytic infiltration.[4] These findings correlate well with measurements of pancreatic insulin extractable at autopsy which indicate a gross lack of endogenous insulin in individuals who acquire the disease during the first twenty years of life.[5]

In the present study, the influence of oral glucose, intravenous tolbutamide, and intravenous arginine on pancreatic insulin secreting capacity of juvenile diabetes has been examined. These studies were performed during each child's initial hospitalization for diabetes mellitus.

METHODS AND MATERIALS

Diabetic subjects: Upon recognition of the diabetic state, fifteen children were hospitalized in the Clinical Research Center of the St. Louis Children's Hospital or at the Research and Educational Hospitals of the University of Illinois. The ages of the children ranged from one to thirteen years. The duration of symptoms ranged from one-half to twelve months. Despite polyphagia, weight loss was documented in nine of the fifteen. The carbohydrate of their diets prior to admission contributed 35 to 50 per cent of their caloric intake. A family history of diabetes was obtained in nine. Admission blood sugar levels ranged from 83 to 770 mg. per 100 ml. Seven patients were ketotic; three were mildly acidotic. Two of the acidotic patients received small doses of crystalline zinc insulin; however, optimal control was not attempted. In these two patients, insulin was withheld for the twelve hours prior to a test. None of the other patients had ever received insulin before testing.

Control subjects. Twenty active healthy nonobese* metabolically normal children served as controls. None had a family history of diabetes. All were well nourished. The carbohydrates of their diets prior to the study contributed 35 to 50 per cent of their caloric intake. The ages of these children ranged from one to thirteen years.

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*No subject exceeded 110 per cent of ideal body weight based on Metropolitan Life Insurance Tables, 1959.

Procedures. Subjects in each group underwent two or more of the following studies: (1) a five-hour oral glucose tolerance test (1.75 gm. per kilogram body weight), (2) a two-hour intravenous tolbutamide tolerance test (20 mg. per kilogram body weight), and (3) an intravenous arginine provocative test (0.5 gm. per kilogram body weight over a thirty-minute period.[6]

All tests were performed after an overnight fast. Venous blood was obtained via an indwelling needle placed in a peripheral vein. The blood was heparinized and kept on ice until sampled for glucose and subsequent centrifugation. Plasma was stored at —14° C. until the time of hormone analysis.

Blood glucose was determined by the glucose oxidase method[7] or on the AutoAnalyzer by the ferricyanide reduction method. Plasma insulin was assayed by the double antibody radioimmunoassay procedure of Morgan and Lazarow.[8] Plasma growth hormone (HGH) was determined by the double antibody radioimmunoassay procedure of Schalch and Parker.[9] The plasma free fatty acid (FFA) was measured by the colorimetric method of Duncombe[10] after a Dole extraction.[11]

Pretreatment sera were tested for the presence of insulin antibodies by the method of Berson and Yalow.[12] Serum was incubated with human insulin labeled with I-131 according to the method of Hunter and Greenwood.[13] Following incubation at 4° C. for forty-eight hours, aliquots were subjected to chromatoelectrophoresis. Controls with nondiabetic sera and with guinea pig anti-insulin sera were run simultaneously. After chromatoelectrophoresis the strips were dried, cut into small segments and counted in a well scintillation auto gamma spectrometer. In addition to the chromatoelectrophoretic technic for separating free and antibody bound I-131-labeled human insulin, two additional procedures were used on several serum specimens: (1) ultracentrifugation at 108,000 × g for eighteen hours—conditions which result in the formation of a well-defined boundary and sedimentation of proteins with molecular weights greater than 60,000, and (2) precipitation of the serum globulins with rabbit antihuman globulin serum.

RESULTS

The means and the standard errors of the means for all constituents measured in blood and plasma during the three provocative tests are shown in table 1.

In the diabetic patient, the initial fasting values for blood glucose at the start of the three tests (means: 192, 245, and 220 mg. per 100 ml.) were almost threefold greater than the corresponding values for the normal controls (means: 74, 78, and 83 mg. per 100 ml.). In spite of the fasting hyperglycemia, neither the initial plasma insulin levels (3-8 μU./ml.) nor the initial plasma growth hormone levels (4-7 mμg./ml.) of the diabetics were significantly different from those of the normal subjects (6-10 μU./ml., 3-5 mμg./ml., respectively). The fasting free fatty acid levels in the diabetics (1,470-1,717 μEq. per liter), however, were almost twice those of the normal subjects (719-853 μEq. per liter).

Plasma insulin response to oral glucose. The blood glucose and plasma insulin responses of fourteen diabetic children and fifteen normal controls are shown in figure 1. In the diabetic patients, marked carbohydrate intolerance was associated with little, if any, pancreatic insulin secretion. Whereas normal individuals achieved a peak mean plasma insulin concentration of 47 μU./ml., the diabetic children failed to exhibit any significant increase—(peak level 10 ± 2 μU./ml.). When the plasma insulin response was related to the corresponding blood glucose level during the course of the tolerance test (expressed as the insulin-glucose ratio), the marked impairment of the insulin secretion became even more evident.

Plasma insulin and glucose response to intravenous tolbutamide. The plasma insulin and blood glucose responses of twelve diabetics and fourteen normal controls are shown in figure 2. In normals, plasma insulin levels rose promptly to a peak of 49 μU./ml. by five minutes with a nadir for blood glucose of 45 per cent of the fasting value at thirty minutes. In the diabetic children, no significant change in the plasma insulin level was observed. Although glucose levels fell by 19 per cent, 120 minutes after tolbutamide injection, none of the changes in blood glucose were significant.

Plasma insulin and glucose response to an arginine infusion. The plasma insulin and blood glucose responses of six diabetic and twelve normal children during and following the infusion of arginine are shown in figure 3. During the arginine infusion there was a slight but consistent rise in blood glucose in both the groups. There was a marked impairment in insulin secretion in the diabetic children with a peak concentration of only 8 μU./ml. compared to 31 μU./ml. for the normal children.

Plasma growth hormone responses (table 1). Following the ingestion of glucose, there was an initial fall with a subsequent rise in the plasma growth hormone level in both normal and diabetic individuals. Tolbuta-

TABLE 1

Insulin, free fatty acid and growth hormone levels during glucose, tolbutamide and arginine tolerance tests

Time minutes	Glucose mg. per 100 ml.	Insulin μU./ml.	FFA μEq./L.	FFA Per cent change	HGH Mμg./ml.	Glucose mg./per 100 ml.	Insulin μU./ml.	FFA μEq./L.	FFA Per cent change	HGH Mμg./ml.
Glucose tolerance test										
	Control (15)*					**Diabetic (14)***				
0	74±5	10±2	853±171	0	5±1	192±25	8±3	1,470±174	0	7±2
30	119±11	30±5	572±71	−23	2±1	311±30	10±2	1,039±94	−29	3±1
60	117±6	47±8	424±73	−50	4±1	411±38	9±2	835±96	−43	1±1
90	106±5	39±10	354±32	−58	4±1	426±37	3±3	776±103	−47	2±1
120	98±7	25±6	343±40	−60	4±1	455±50	7±2	768±103	−48	6±2
180	76±5	14±6	451±113	−47	5±1	394±43	6±2	872±88	−41	5±1
240	70±4	8±2	639±123	−25	12±2	287±36	7±2	1,030±97	−30	6±1
300	79±7	9±2	1,163±145	+37	7±2	269±36	5±2	1,237±140	−16	3±1
Tolbutamide tolerance test										
	Control (14)*					**Diabetic (12)***				
0	78±2	7±1	847±171	0	5±1	245±37	3±1	1,717±176	0	4±1
5	72±2	49±7	734±103	−13	8±2	238±22	5±1	1,922±276	+11	4±1
10	63±3	44±10	728±161	−14	7±1	237±38	5±1	1,453±128	−15	3±1
20	53±3	28±6	502±101	−41	4±1	238±40	4±1	1,229±119	−28	4±1
30	50±4	18±3	454±65	−46	4±1	227±43	4±1	998±121	−42	4±2
45	52±3	13±1	620±145	−27	6±1	208±34	3±1	1,027±106	−40	6±1
60	58±3	9±2	774±194	−9	10±4	202±29	3±1	1,106±114	−36	5±1
90	63±2	10±1	890±229	+2	8±2	213±18	7±2	1,297±191	−25	3±1
Arginine tolerance test										
	Control (12)*					**Diabetic (6)***				
0	83±3	6±2	719±79	0	3±1	220±26	4±2	1,521±425	0	4±2
15	101±10	20±5	606±88	−17	8±3	232±28	8±3	1,132±256	−26	9±3
30	100±11	30±8	439±36	−39	17±3	240±32	9±3	1,014±298	−33	17±6
45	92±4	18±5	394±19	−42	24±4	260±40	6±3	915±253	−40	16±5
60	84±5	16±4	530±107	−26	22±4	240±38	6±3	1,113±399	−26	18±4
90	86±6	5±2	869±70	+21	15±3	230±34	5±2	1,292±362	−13	18±3
120	88±4	3±2	—	—	8±2	220±30	5±2	—	—	12±5

*Figures in parentheses indicate number of subjects.

mide injection produced no predictable or significant change in HGH concentration in either group. Arginine infusion resulted in a prompt four- to eightfold increase in plasma HGH concentration in both groups. There were no significant differences between the plasma HGH responses of the diabetic and the control groups to these three provocative tests.

Plasma free fatty acid response (table 1). Although the fasting plasma FFA levels were higher in the diabetic than in the control subjects, the FFA responses following oral glucose, intravenous tolbutamide and arginine infusion, when expressed as a per cent change from the baseline values, were equivalent to those seen in normal individuals.

Insulin antibodies. The chromatoelectrophoretic migration on Whatman 3M filter paper of I-131-labeled human insulin incubated with diabetic sera was the same as that seen when the labeled hormone was incubated with sera obtained from normal nondiabetic individuals. (table 2). Furthermore, no insulin antibodies were detected in diabetic sera by either immunoprecipitation of the globulin fraction with antihuman globulin rabbit serum or by ultracentrifugation. Insulin antibody was readily detected by all three procedures in sera obtained from three diabetic subjects on long-term insulin therapy.

DISCUSSION

Juvenile diabetes mellitus has been thought to represent a state of absolute insulin deficiency in contrast to the maturity-onset type diabetes mellitus in which there may be insulin antagonism and a resulting compensatory hypersecretion of insulin.[14,15] It has been suggested that juvenile insulin-dependent diabetics, early in the course of their disease, may go through a hyperactive stage characterized by postprandial hyperinsulinism and subsequent hypoglycemia.[16] If such a sequence of events does occur, the children observed during the course of this study had progressed to the point of pancreatic beta cell decompensation which resulted in little,

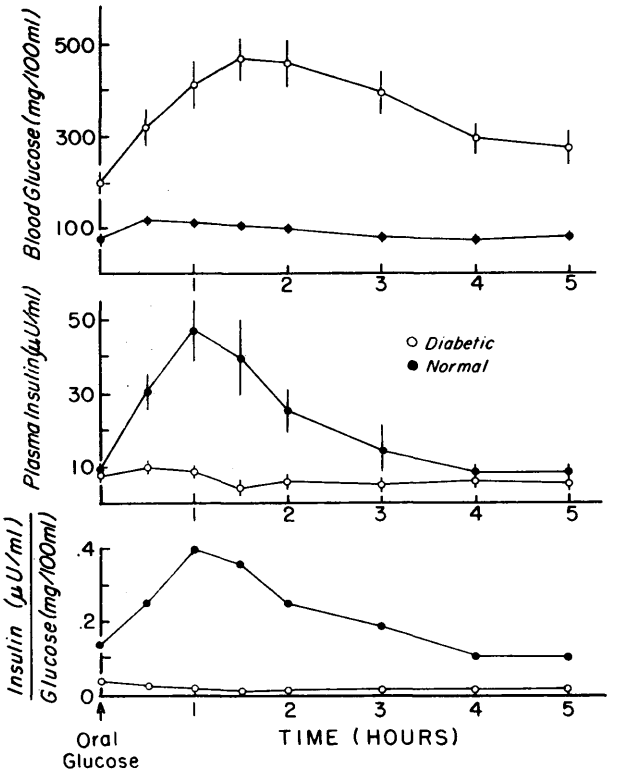

FIG. 1. Blood glucose and plasma insulin responses to oral glucose in fifteen normal and fourteen juvenile diabetic subjects. Values represent mean ± S.E.M. In the lower portion of the figure, the plasma insulin response is expressed as the index of plasma insulin/blood sugar.

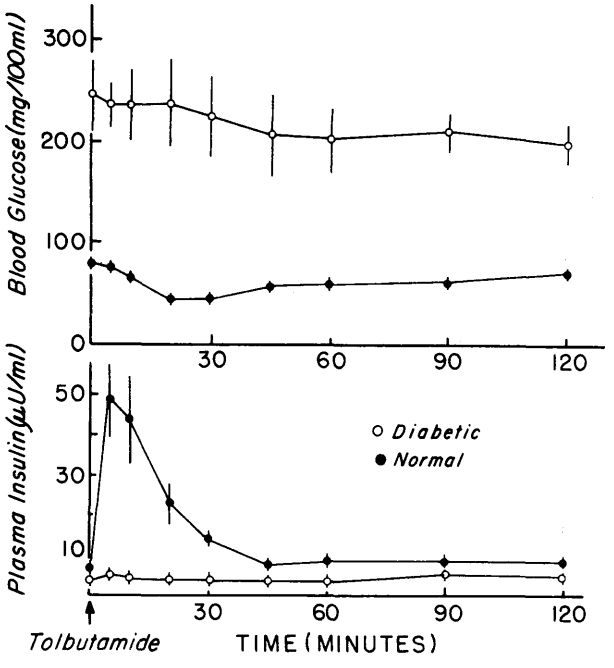

FIG. 2. Blood glucose and plasma insulin response to intravenous tolbutamide in fourteen normals and twelve juvenile diabetics. Values represent mean ± S.E.M.

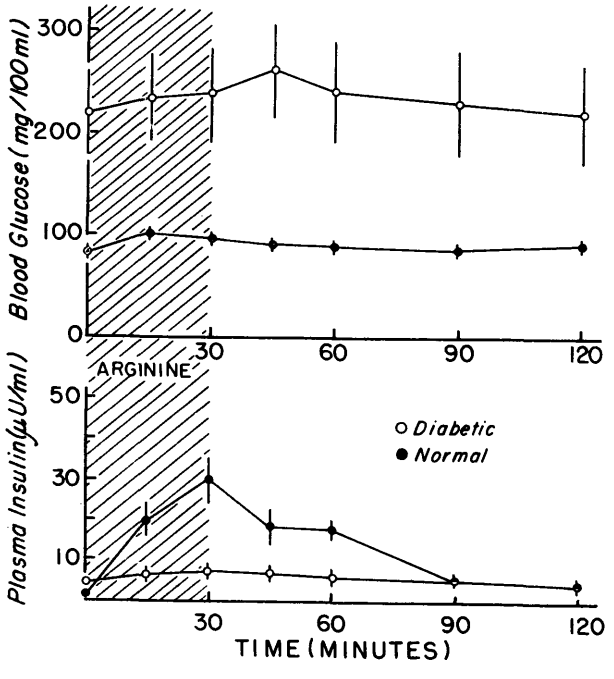

FIG. 3. Blood glucose and plasma insulin in response to a thirty-minute infusion of arginine in twelve normal and six juvenile diabetic subjects. Values represent mean ± S.E.M.

if any, change in plasma insulin levels following a variety of insulinogenic stimuli.

Fasting plasma insulin values in our normal subjects are in agreement with the adult values reported by Perley and Kipnis[17] although lower than those of Ehrlich and Bambers.[3] The most likely explanation for this discrepancy is in the sensitivity of the technic employed. The immunoassay procedure as used in our laboratories permits maximum precision between 10 to 100 μU./ml., but is sensitive to 1.0 μU./ml. of plasma. The mean fasting plasma insulin values for the diabetic subject and the control subjects were the same; however, the corresponding blood sugar levels were much higher in diabetic children, and the insulin/glucose ratios were significantly lower. During the glucose, tolbutamide, and arginine provocative tests, no significant rise in the patients' serum insulin occurred; the normal individual exhibited a brisk rise in serum insulin following each of the three stimuli.

There is an increased incidence in the onset of symptomatic diabetes at the time of the pubescence growth

TABLE 2

The distribution of human insulin-I-131 in serum of normal and newly diagnosed juvenile diabetics and diabetics on long-term insulin therapy

	Per cent distribution of radioactivity					
	Chromatoelectrophoresis		Ultracentrifugation		Globulin Precipitation	
Patients	Free	Bound	Supernatant	Protein Layer	Supernatant	Precipitate
Juvenile diabetics						
1	94	6	35	65	88	12
2	88	12	30	70	90	10
3	90	10	36	64	96	4
4	95	5	40	60	92	8
5	92	8	35	65	85	15
6	91	9	32	68	88	12
Control						
1	95	5	36	64	88	12
2	90	10	30	70	93	7
3	92	8	37	63	91	9
Long-term insulin therapy						
1	36	64	4	96	25	75
2	25	75	12	88	20	80
3	26	74	8	92	32	68

spurt. The possibility arises that growth hormone secretion might be increased at this time and contribute, in view of its human insulin antagonistic effect, to this increased incidence of diabetes. Our studies show that the fasting plasma growth hormone concentrations were the same in diabetic and control subjects and that there was no significant difference between plasma growth hormone response of the diabetic and the normal individual under the conditions of these experiments. There remains the possibility, however, that intrinsically regulated growth hormone secretion might be elevated at the time of a growth spurt, but that neither the basal level nor the response to an arginine provocative test reflects this change.

An explanation for the higher mean fasting plasma FFA concentration in diabetic children in comparison to the normal subjects is not immediately apparent. Although our values for normal children are lower than those reported by Davidson,[18] it should be pointed out that the FFA level is extremely variable in normal children and in our group ranged from 475 μEq. per liter to 1,360 μEq. per liter. In the diabetic patients the range was 824 μEq. per liter to 2,795 μEq. per liter. One possible explanation for the fasting FFA differences is that the mean fasting insulin level in diabetes is less than in normals and that our immunoassay procedure did not have sufficient precision to distinguish significant differences at these low levels of plasma insulin. The same cannot be said for the failure to detect differences in the growth hormone levels in these subjects, since these levels were within the range of maximal precision of the immunoassay system.

A substantial decrease in the free fatty acid level occurred in both diabetic and control subjects following either glucose ingestion, intravenous tolbutamide or arginine infusion. Indeed, when expressed in terms of per cent decrease from baseline values, no distinction was evident in the FFA response of these two groups. The lipolytic system of the fat cell is exquisitely sensitive to insulin and concentrations as small as 1 μU./ml. have been reported to inhibit the release of free fatty acids and glycerol from adipose tissue.[19,20] Since small amounts of insulin were present in our growth-onset diabetic patients, slight increases, sufficient to inhibit lipolysis but not adequate to alter glucose tolerance, could occur which would not be detected by radioimmunoassay of the plasma insulin concentration.

It would seem more likely, however, that a variety of factors other than insulin alone are responsible for the FFA responses noted in these patients. For example, it has been shown that the rate of glucose uptake and fatty acid re-esterification by adipose tissue is directly proportioned to the extracellular glucose level over a wide range of physiological concentrations.[21,22] As a consequence, even under conditions of hormone stimulated lipolysis, FFA release can be diminished by raising the extracellular glucose level and accelerating fatty acid re-esterification. Thus, the fall in the FFA level seen in the normal subject following glucose ingestion may reflect primarily an insulin mediated inhibition of lipolysis,[23] whereas in the diabetic individual it may be subsequent to accelerated re-esterification secondary to

the marked increase in circulating glucose. In the case of tolbutamide, Stone et al.[24] have recently reported that this agent, at levels comparable to those attained in the human receiving the usual pharmacologic dose, exerts a direct inhibitory effect on adipose tissue lipolysis independent of its known insulinogenic activity. Lastly, the fall in FFA seen following arginine infusion may reflect a combination of the inhibitory action of insulin on adipose tissue lipolysis and an acceleration of FFA utilization which several workers have recently reported to be an acute effect of growth hormone[15,25,26] in contrast to its delayed adipokinetic activity.

It has been suggested that autoimmune mechanisms may be involved in the pathogenesis of diabetes mellitus in at least two ways: (1) the development of insulin antibodies would produce a form of insulin antagonism ultimately leading to islet cell exhaustion, and (2) the presence of antibodies specific to some cellular component of the beta cell other than insulin could lead to islet cell destruction and insulin deficiency. Our studies are pertinent to the first mechanism. Despite use of a variety of technics, neither insulin antibodies nor other abnormal insulin binding materials were detected in the sera of newly diagnosed growth-onset diabetics.

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