Glucose Tolerance and Serum Insulin in Unaffected Identical Twins of Diabetics

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Since diabetes mellitus is partly inherited a study of the unaffected identical twin of a diabetic may yield useful information regarding both its genetics and its metabolic abnormalities.

About half of the pairs of identical twins so far reported have been concordant—that is, both twins were diabetic (Joslin *et al.*, 1959; Harvald and Hauge, 1963). If diabetes is transmitted by a single gene then all the twins ought eventually to become diabetic, unless "penetrance" is incomplete. Glucose-tolerance tests may reveal abnormalities in such apparently unaffected twins.

In addition the insulin response to glucose of a series of twins of diabetics has not so far been reported. Such studies might reveal whether these apparently normal twins show antagonism to insulin or impairment of the response of the beta cell to glucose. Thus increased blood insulin levels with normal glucose tolerance might indicate antagonism to insulin, and lower levels after glucose might reflect a diminished insulin secretion.

We report here the results of glucose-tolerance tests performed on nine apparently healthy identical twins of diabetics. Blood insulin was determined during the tolerance tests.

Present Investigation

Subjects.—The clinical characteristics of the twins are shown in Table I. In six cases the twins were female and in three male. The age at diagnosis of diabetes in the affected twin varied from 5 to 52; in all but two it was under 45 years. The mean interval from diagnosis in the affected twin to the time of testing the unaffected twin was five years. In pair 3 both twins were fat (50% overweight), but in the other pairs neither the diabetic nor the non-diabetic twin was significantly overweight—that is, none was 10% overweight. In seven of the nine pairs there was a family history of diabetes; in pair 2 both parents were diabetic.

TABLE I.—*Clinical Details of Identical Twins Studied*

Pair No.	Sex	Age at Test	Age at Diagnosis of Diabetes in Affected Twin	Familial Relationship with Other Members of Family with Diabetes
1	F	23	13	Grandfather, 2 great aunts, 1 uncle. (All maternal)
2	M	55	52	Mother. Father. Daughter
3	M	55	50	Father
4	F	23	20	None
5	M	30	28	"
6	F	43	43	Uncle
7	F	43	43	Mother
8	F	56	44	Sister
9	F	14	5	Great aunt. Great uncle (maternal)

Normal Persons.—The normal group for comparison of serum insulins consisted of 24 individuals (8 male, 16 female) aged between 18 and 75. None of the normals differed from their expected weight by more than 10% and none was known to have a family history.

Procedure.—In some cases the tests were done at King's College Hospital, and in others one of us carried out the test in the patient's home. All subjects took a full carbohydrate diet for at least three days before the test. Venous blood was taken from a forearm vein after an overnight fast. Then 50 g. of glucose was given by mouth, and blood samples were taken 30, 60, and 120 minutes later. Determinations were made of glucose and insulin. For determination of insulin the blood was allowed to clot and the serum was separated by centrifugation. Serum was stored at −20° C. Insulin was measured by immunoassay; Hales and Randle's (1963) method being used with certain modifications, the most important being the use of 0.01 M disodium diethyltetracetic acid in the reaction mixture (Morgan *et al.*, 1964; Sheldon and Taylor, 1965), to avoid interference from complement. Results on human serum were compared with the response to ox insulin standards prepared from a recrystallized specimen kindly provided by Wellcome Laboratories, Beckenham, Kent. Glucose was determined by an oxidase method (Huggett and Nixon, 1957).

Confirmation that twins were identical was obtained by determining blood groups ABO, MN, S, P, Lu^a, Le^a, Le^b, Fy^a (through the kind help of the Blood Group Reference Laboratory, Lister Institute).

Results

Normal Persons.—Blood glucose and serum insulin levels in normal persons are shown in Table II. These results do not show marked differences from those of many other workers who have employed a test of this type. The blood glucose results, for example, are very slightly lower than those of Welborn *et al.* (1966) for 45 normal volunteers in whom blood glucose was determined by a ferricyanide method after 50 g. of oral glucose. Serum insulin values obtained by various immunoassay methods which differ considerably in detail have been remarkably uniform from one group of workers to another (see Taylor, 1967). Moreover, our results in normal subjects differ only slightly from values in an earlier series obtained in 10 normal people in this department (Rudnick and Taylor, 1965). As has been noticed by Welborn *et al.* (1966) serum insulin levels did not differ with age.

TABLE II.—*Glucose Tolerance and Serum Insulins in Unaffected Identical Twins of Known Diabetic Patients*

	Glucose Tolerance (mg./100 ml.)				Insulin (μu./ml. Serum)			
	0 min.	30 min.	60 min.	120 min.	0 min.	30 min.	60 min.	120 min.
Normals*	67 ± 2	98 ± 4	80 ± 3	67 ± 3	21 ± 2	64 ± 5	57 ± 6	31 ± 6
Patient:								
1	77	133	137	118	24	65	63	53
2	80	162	183	125	9	22	29	22
3	95	145	162	125	27	26	50	50
4	67	71	65	55	10	11	16	13
5	78	114	59	63	29	100	45	11
6	94	147	154	71	13	41	43	18
7	76	145	185	123	—	16	32	2
8	86	129	136	38	11	14	10	11
9	92	122	163	110	18	25	26	6
Mean ± S.E.M.	83 ± 3	133 ± 10	137 ± 16	92 ± 4	18 ± 3	36 ± 10	35 ± 5	21 ± 6

* Thirty-four normal individuals were tested for glucose tolerance and 25 for serum insulin.

Twins.—Serum insulin and blood glucose values in the nine twins of diabetics are shown in Table II. It will be seen that

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glucose tolerance is abnormal by comparison with the control group. Blood glucose levels are significantly different in the twins from those in the controls in the fasting state and after glucose. In only two curves did all the points lie within two standard deviations of normal values. The others showed high values, especially one and two hours after glucose. The two subjects with normal glucose tolerance results were from the only pairs without a family history of diabetes. Serum insulin levels in the fasting state did not differ between the groups. However, insulin levels were lower than normal half an hour and one hour after glucose in the twins.

Discussion

The significance of these abnormal blood glucose levels is increased by the fact that the subjects are young. High blood glucose values after oral glucose are rare in early life (Butterfield, 1964). Despite these high blood glucose levels serum insulin levels are low, particularly after glucose.

There are a number of possible explanations for this reduced response to glucose. It might be that not all the insulin in the blood is being measured. At present there is no conclusive evidence to suggest that immunological methods fail to measure all the available insulin in the blood. When attempts are made to extract insulin from blood proteins by various acid-ethanol methods there does not appear to be any release of insulin previously associated with other proteins (Karam *et al.*, 1963). When higher values for blood insulins have been obtained by bioassay methods their significance is doubtful. It has always been difficult to measure blood insulin levels with precision by the rat diaphragm technique (Taylor and Randle, 1959), and the biological significance of adipose tissue assays for blood insulin is uncertain (Kipnis and Stein, 1964; Rasio *et al.*, 1965).

It might be that insulin was being more rapidly broken down in the twins of diabetics. However, serum insulin levels tend to rise after glucose less than in normal persons, though fasting levels are not different in the two groups. This is not the pattern of response which would be expected if there were a uniformly enhanced rate of insulin destruction throughout the glucose-tolerance test. Neither do these studies give any evidence of antagonism to endogenous insulin, which would lead to raised levels of blood insulin in the presence of a normal glucose tolerance. It seems much more likely that the response of the beta cell to a rise of glucose concentration is less than normal. The diminished glucose tolerance of some of these non-diabetic twins may be a consequence of diminished insulin response to oral glucose.

The clinical significance of these findings is not known. In particular, we do not know whether the abnormalities will progress to frank clinical diabetes. We intend to observe the present group and collect data on other identical twins of diabetics.

Summary

Glucose tolerance and serum insulin levels have been examined in nine apparently normal twins of diabetics and compared with the results in normal people.

Mean glucose tolerance was significantly less than that of normals and serum insulin levels after sugar were also low.

It is suggested that the impairment of glucose tolerance in some of these twins is a consequence of a diminished response of the beta cell to glucose.

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Glucose Tolerance and Serum Insulin in the Unaffected First-degree Relatives of Diabetics

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In the preceding paper the effects of oral glucose on blood glucose and serum insulin levels were examined in the identical twins of known diabetics, and abnormalities of glucose tolerance and serum insulin levels in some of them were shown. It seemed of interest to determine whether these abnormalities were present in other first-degree relatives of diabetic patients. In addition, serum insulin and glucose tolerance were examined in a small group of relatives of diabetic patients who gave clinical evidence of hypoglycaemia.

Methods and Subjects

Blood glucose and serum insulin were measured by methods identical to those in the preceding paper. The results have been compared with the same control series.

Six children of conjugal diabetic couples were studied and studies were made of a family of nine children derived from an additional conjugal pair of diabetics.

Nine other first-degree relatives of diabetics were studied. None of them was previously known to have diabetes. As in the preceding paper, subjects were tested after an overnight fast and after at least three days on a normal carbohydrate diet. All but one of these patients were aged under 45.

Results

Values for Glucose and Insulin in Relatives of Diabetics.—When oral glucose-tolerance tests were performed in the off-

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