Diabetes Mellitus in Gonadal Dysgenesis:
Studies of Insulin and Growth Hormone Secretion

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Abstract. On the basis of results obtained from an oral glucose tolerance test (OGTT), twenty patients with gonadal dysgenesis were classified as normal (N = 8) and diabetic (N = 12). The two groups of patients were further tested by a rapid intravenous glucose injection, a tolbutamide test, an insulin sensitivity test and an oral amino acid load. Fasting levels of plasma growth hormone (GH) were normal in all subjects but one. Approximately 1/3 of the GH responses during testing periods were abnormal, being either absent during hypoglycaemia or following amino acid ingestion, or paradoxically increased during hyperglycaemia. No correlation was found between the degree of carbohydrate intolerance and the levels of plasma GH. There was no gross alteration of tissue sensitivity to exogenous insulin. The β-cell response to tolbutamide, amino acid and intravenous glucose were comparable in patients with a normal or a diabetic OGTT. In both groups, the rates of decrease of blood glucose following tolbutamide or intravenous glucose were also similar and within the normal range. During OGTT, the diabetic group had a delayed insulin release and a low insulinogenic index. It is concluded that in gonadal dysgenesis the intolerance to an oral carbohydrate load is frequently associated with, but unrelated to, anomalies of the GH secretion. In diabetic subjects, the process of insulin secretion loses its normal sensitivity to the oral glucose stimulus while remaining unaltered and similar to that of non-diabetic subjects in response to tolbutamide, amino acid and intravenous glucose.

Key words: Gonadal dysgenesis, Turner's syndrome, amino acid, tolbutamide, oral and intravenous glucose, growth hormone, insulin.

Gonadal dysgenesis is frequently associated with carbohydrate intolerance (Forbes and Engel 1963; Menzinger et al., 1966; Jackson et al., 1966; Nielson et al., 1969; Van Campenhout et al., 1973). Whether such an anomaly represents true diabetes mellitus or not is still a matter of controversy: few patients have been investigated, usually by a limited series of tests. The carbohydrate intolerance has been ascribed to elevated fasting concentrations of growth hormone (Lindsten et al., 1967) and or- to paradoxical stimulation of growth hormone secretion during hyperglycaemia (Lindsten et al., 1967; Nielsen et al., 1969). These proposals have been disputed (Meadow et al., 1968).

To further evaluate the beta-cell function in gonadal dysgenesis and to determine the possible effect of growth hormone secretion anomalies on glucose tolerance, a series of twenty patients was investigated: on the basis of tolerance to oral glucose, eight classified as normal and twelve as diabetic. Both groups were submitted to a rapid intravenous glucose injection, a tolbutamide test, an insulin sensitivity test and an oral amino acid load. The interrelationships between plasma levels of glucose, amino acid, insulin and growth hormone were compared in the two groups.

Material and Methods

Twenty patients with gonadal dysgenesis were investigated. The diagnosis of gonadal dysgenesis was based on clinical, biological, cytogenetical and histopathological criteria, as already reported (Van Campenhout et al., 1973). Thyroid function (protein bound iodine, tri-iodothyronine, thyroxine, thyroid uptake of radioactive iodine, thyroid scanning), and adrenal function (plasma cortisol, urinary cortisol and 17 ketoestroids, metyrapone test) were normal in all patients. Tomographies of the sella turcica did not show radiological anomalies. Visual activity and fields and retinal vessels were normal. There were no clinical or laboratory abnormalities of the hepatic and renal functions.

The patients were divided into two groups on the basis of a normal or a diabetic oral glucose tolerance curve. The group referred to as normal comprised 8 subjects; the other 12 subjects had fasting blood sugar levels within the normal range but their hyperglycaemic profiles complied with the diagnostic criteria of the United States Public Health Service (Remain and Wilkerson, 1961) and of the Joslin Clinic (Marble, 1971) for diabetes mellitus. The two groups were comparable with respect to average
Table 1. Clinical data on patients with gonadal dysgenesis

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean age</th>
<th>Mean height</th>
<th>Mean weight</th>
<th>Familial diabetes</th>
<th>Oral contraceptives</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>no.</td>
<td>years cm</td>
<td>kg</td>
<td></td>
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<tr>
<td>Normal OGTT</td>
<td>8</td>
<td>25 (17-34) 149 (135-165)</td>
<td>45 (37-52)</td>
<td>2 (25 %)</td>
<td>5 (63 %)</td>
</tr>
<tr>
<td>Diabetic OGTT</td>
<td>12</td>
<td>24 (14-38) 145 (131-170)</td>
<td>50 (35-67)</td>
<td>8 (67 %)</td>
<td>8 (67 %)</td>
</tr>
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</table>

height, weight, age, and frequency of oral contraceptive hormones intake. One patient in the diabetic group was overweight by 20% and one patient in the non-diabetic group had an excessive weight of 10% (by reference to the desirable weights of the Metropolitan Life Insurance Company). All other subjects had body weights in the ideal range. Patients on oral contraceptives were taking oestrogen and progesterone for more than 18 months; one week prior to testing, the hormonal treatment was discontinued. A positive history of familial diabetes (at least one relative in 2 generations) was reported by 8 of the 12 diabetic patients and by 2 of the 8 normal patients (Table 1). The investigations were carried out at the Hospital Metabolic Unit, where all patients had been admitted and given a high calorie (approximately 2000 Cal/day), high-carbohydrate (200 - 300 g/day) diet for 3 days prior to testing. The same diet was maintained throughout the stay at the hospital. The following tests were performed, each after an overnight fast and with the patient lying quietly in bed: (1) 100 g of glucose dissolved in 200 ml of flavoured water were given orally over approximately 5 minutes (OGTT); (2) 0.33 g of glucose per kg body weight in the form of a 20 g/100 ml water solution were administered into an antecubital vein in exactly 3 minutes (IVGTT); (3) 1 g of sodium tolbutamide (IVTT) and (4) 0.1 U/kg body weight of crystalline insulin (IVIT) were rapidly injected by the intravenous route; (5) the oral amino acid tolerance test consisted of the ingestion of 75 g of casilan powder in water, over a 30 minute period of time (OATT); only 15 patients were submitted to this test, 9 of whom were diabetic and 6 normal. The composition of the casilan powder is given in Table 2. All tests were carried out on separate days, in random sequence.

Approximately 30 minutes prior to testing, a catheter was inserted into an antecubital vein which was maintained patent by the infusion of small amounts of physiological saline. Blood samples were timed from the start of injection or the first swallow of glucose solution. However, for the OATT, they were timed from the end of the 30 min. period of casilan ingestion. Plasma glucose was measured by the method of Brown (1961) adapted to a Technicon Autoanalyser. The net rate \( K \) of glucose utilization during IVGTT, was calculated according to the equation

\[
K = \frac{0.693}{t_1/2}
\]

where \( t_1/2 \) is the half-time of the slope of log glucose concentrations over time, between 10 and 50 minutes (Conard, 1955). Blood amino acid concentrations were determined by the method of Frame et al. (1943). Plasma insulin (IRI) and growth hormone (GH) levels were measured in duplicate by double antibody radio-immunoassay techniques (Soeldner and Slone, 1965; Boden and Soeldner, 1967), using as standards, crystalline bovine insulin (lot No 0 12269, 25.8 IU/mg, courtesy of Dr. J. Schlichtkrull, Novo Laboratories, Copenhagen) and purified human GH (lot No NIH-GH-HS 1394, 2.0 IU/mg, courtesy of Dr. E.A. Wilhelm, Emery University, Atlanta, Georgia, through N.I.H., Bethesda). Any abnormal response of GH was

<table>
<thead>
<tr>
<th>Mean percentage</th>
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<tbody>
<tr>
<td>Leucine 8.8</td>
</tr>
<tr>
<td>Isoleucine 6.0</td>
</tr>
<tr>
<td>Lysine 7.3</td>
</tr>
<tr>
<td>Methionine 2.8</td>
</tr>
<tr>
<td>Phenylalanine 4.8</td>
</tr>
<tr>
<td>Threonine 4.0</td>
</tr>
<tr>
<td>Tryptophan 1.1</td>
</tr>
<tr>
<td>Valine 6.3</td>
</tr>
<tr>
<td>Arginine 3.7</td>
</tr>
<tr>
<td>Cystine 0.3</td>
</tr>
<tr>
<td>Glycine 2.0</td>
</tr>
<tr>
<td>Histidine 2.8</td>
</tr>
<tr>
<td>Tyrosine 5.8</td>
</tr>
<tr>
<td>Alanine 2.9</td>
</tr>
<tr>
<td>Aspartic acid 6.4</td>
</tr>
<tr>
<td>Glutamic acid 20.1</td>
</tr>
<tr>
<td>Proline 10.3</td>
</tr>
<tr>
<td>Serine 5.5</td>
</tr>
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Calorific value 108 per oz.
Fig. 1. Insulin and growth hormone levels in plasma during oral glucose tolerance test (OGTT) in patients with gonadal dysgenesis. n = number of patients. Solid line: patients with normal oral glucose tolerance. Dotted line: patients with diabetic oral glucose tolerance. Mean values ± S.E.M.

verified by repeating and reassaying the test.

The insulinogenic index is the ratio of insulin (µU/ml) over glucose (mg/ml) increments above corresponding baseline values at any given time of a glucose test (Seltzer and Smith, 1959).

Results

1) Oral Glucose Tolerance Test (Fig. 1). In the diabetic group, the insulin response is blunted and delayed: a peak value of 85 µU/ml is reached at 120 min. as opposed to a peak value of 117 µU/ml at 30 min. in the non-diabetic group. The plasma insulin concentrations when related to the corresponding plasma glucose levels are consistently lower in the diabetic subjects: their average insulinogenic index from the 30th minute onwards is 25% of the index calculated in the non-diabetic subjects (P < 0.001). Mean plasma GH values before and after glucose ingestion are comparable in both groups and fluctuate between 2 and 5 ng/ml. However, 3 patients out of 12 in the diabetic group respond by a persistent rise of plasma GH to values between 10 and 20 ng/ml during the first hour of the test, while none does so in the non-diabetic group (Table 3).

2) Intravenous Glucose Tolerance Test (Fig. 2). The net rate K of glucose utilization averages 1.73 ± 0.15 x 10^-2 per min. in non-diabetics and 1.57 ± 0.09 x 10^-2 per min. in diabetics (P > 0.05). The insulin responses are rapid. Although
Fig. 2. Insulin and growth hormone levels in plasma during intravenous glucose tolerance test (IVGTT) in patients with gonadal dysgenesis. n = number of patients. Solid line: patients with normal oral glucose tolerance. Dotted line: patients with diabetic oral glucose tolerance. Mean values ± S.E.M.

The insulin values are consistently higher in diabetic subjects, the insulinogenic indexes are comparable in both groups (P > 0.05), at any given time between 0 and 50 min. and vary between 30 and 70 µU/ml.

GH levels in plasma fluctuate between 2 and 8 ng/ml. In 2 of 6 non-diabetics and in 4 of 12 diabetics, GH concentrations rise to values between 10 and 40 ng/ml before the 40th min., for at least two consecutive sampling times (Table 3).

3) Intravenous Tolbutamide Test (Fig. 3). During the first 15 minutes of the test, the percentage drop of plasma glucose concentrations is higher in non-diabetic than in diabetic subjects (P < 0.05). In all patients, however, fasting blood sugar declines by more than 40% at the 30th minute.

The patterns of insulin response are comparable.

With the exception of one patient whose plasma GH levels remain steady throughout the test, all other subjects in the non-diabetic group respond by rises of plasma GH concentrations varying between 8 and 50 ng/ml during the tolbutamide induced hypoglycaemia. The GH responses in the diabetic patients are either absent (4 subjects) or sporadic and not exceeding 18 ng/ml (8 subjects) (Table 3).

4) Insulin Sensitivity Test (Fig. 4). The rates of decrease of plasma glucose concentrations are comparable in the two groups (P > 0.05) with a nadir at 30 min. representing 40% of the fasting value.

The GH responses vary between 8 and 55 ng/ml in non-diabetics and between 8 and 50 ng/ml in diabetics. Three of the 8 non-diabetics and 4 of the 12 diabetics do not respond by a rise of plasma GH levels (Table 3).

5) Oral Amino Acid Tolerance Test (Fig. 5). In both groups, plasma glucose levels rise by 10% (P < 0.05) and amino acid concentrations by 70% (P < 0.01) from 15 to 180 min. The insulin responses are brisk and similar.
The GH responses vary between 8 and 16 ng/ml in non-diabetics and between 10 and 42 ng/ml in diabetics.

Three among the 9 diabetic subjects tested and one among the 6 non-diabetic subjects do not show any significant change in plasma GH concentration (Table 3).

Discussion

1) Growth Hormone Secretion. It has been proposed that high levels of GH during fasting (Nielsen et al., 1969) or following glucose administration (Lindsten et al., 1967; Nielsen et al., 1969) are responsible for the carbohydrate intolerance observed in patients with gonadal dysgenesis. Although our results do not warrant these specific assumptions, they clearly demonstrate a widespread malfunction of the mechanisms involved in the secretion of GH.

In the 90 tests performed, there are 25 abnormal responses of GH. No correlation is found between either the type of gonadal dysgenesis or the history of oral contraceptive intake and the frequency of abnormal GH responses whether determined on the per test or per patient basis. In Table 3, it can be seen that if all patients on oral contraceptives are assembled, regardless of their glucose tolerance, there are 15 abnormal responses of GH for 61 tests in 13 patients, as opposed to 10 abnormal responses of GH for 29 tests in 7 patients without oral contraceptives. The lack of relationship between oestrogen intake and frequency of GH aberrant responses also applies if only the stimulatory tests are considered.

Although normal subjects may occasionally behave as non-responders (Raiti et al., 1967) hyposecretion of GH during hypoglycaemia and following protein ingestion is unusually frequent and accounts for approximately 2/3 of the anomalous responses; hypersecretion of GH during hyperglycaemia makes up the balance. One patient with chemical diabetes has paradoxical changes of plasma GH levels in all tests (Fig. 6); this patient has elevated baseline GH concentrations prior to insulin, tolbutamide and amino acid administration. Under these conditions, a decreased secretion of GH in response to stimuli is expected (Frohman et al., 1967), presumably because GH suppresses its own secretion in a feedback mechanism (Sakuma and Knobil, 1970).

For reasons which are not apparent, the disturbances in the dynamics of GH secretion are more frequent in diabetic than in non-diabetic subjects, on all counts of comparisons (Table 3). The question arises as to what extent the hypersecretion of GH can play a pathogenic role in the induction of diabetes in gonadal dysgenesis. It should first be emphasized that plasma GH levels in all subjects, during fasting, fall in the range of values reported in the literature or observed in our laboratory for normal female subjects; indeed when values at -10 and 0 min. from all tests are pooled, they average 3.1 ±
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Irrespective of the group investigated, insulin hypoglycaemia is as rapid and as pronounced as the one reported in normal subjects following the injection of weight related amounts of insulin (Creutzfeldt et al., 1962).

It is concluded that sporadic anomalies of the GH secretion are frequently encountered in patients with gonadal dysgenesis. Occasionally, a systematic aberrant secretion can be detected. These anomalies do not correlate with the degree of carbohydrate intolerance.

2) Insulin Secretion. In idiopathic diabetes there is a relative or absolute diminution of insulin secretion in response to tolbutamide, amino acid and glucose stimulations. Diabetes in gonadal dysgenesis seems characterized by a more selective defect of the beta-cell function. Whether this phenomenon is related to specific sex chromosome anomalies in gonadal dysgenesis or is the expression of a limited number of the many genetic factors responsible for idiopathic diabetes, is a matter of speculation. The latter hypothesis would conform with the lack of correlation between the type of gonadal dysgenesis and glucose tolerance in our series and with the higher frequency of idiopathic diabetes among relatives of our group with oral glucose intolerance.

Among the 20 patients tested with intravenous tolbutamide, not one would be termed diabetic by the accepted standard of the percentage fall of fasting blood glucose level (Unger and Madison, 1958). The sensitivity of the beta-cell to the sulphonylurea is similar in both groups of patients, irrespective of their oral glucose tolerance. The discrepancy in the beta-cell capacity to respond to tolbutamide and glucose in the diabetic group is consonant with the fact that these stimuli involve different mechanisms of insulin secretion (Malaisse, 1968).

Following a protein meal, there is a 70% increase in the level of total plasma amino acid nitrogen and a 10% rise in plasma glucose concentration; these changes are noticed 15 min. after the patients have completed the ingestion of casilasan and are maintained throughout the 3 hours of the test. Again, as for tolbutamide, the insulin responses in the two groups are superimposable. It is of interest that our results match those reported by Berger et al. (1964) and Floyd et al. (1966) on healthy subjects of similar age, fed lean beef or chicken liver and where the glycaemic and amino acid profiles were comparable to those of our patients. Although after ingestion of casilasan the levels of individual amino acids in plasma may have been different from those obtained after a meat meal, it is possible that in gonadal dysgenesis, patients with an abnormal tolerance to oral glucose have a normal tolerance and a normal beta-cell sensitivity to oral amino acids.

A clear disturbance which emerges from this study is related to glucose tolerance. Following the oral administration of glucose, 8 patients...
behave as normal and 12 as diabetics. Although the standard dose of 100 g of oral glucose may seem excessive in individuals who weigh 50 Kg, we have shown that it is well tolerated by healthy female controls of the same weight and who respond with normal hyperglycaemic profiles (Van Campenhout et al., 1973). In the diabetic group, the insulin response is delayed and the insulinogetic index is considerably reduced from 30 to 180 min. One common explanation for this phenomenon is that the process of insulin secretion by the beta-cell has lost its normal sensitivity to the glucose stimulus.

Following the intravenous injection of glucose, the net rate K of glucose utilization is 1.57 in the diabetic group and 1.73 in the non-diabetic group. These values are not significantly different; although they seem low for patients, such as ours, in the age class of 20 - 30 years (Kahn et al., 1967; Rasio 1971) they are clearly above the diabetic range (Conard et al., 1971). Moreover, in the diabetic group, the insulin response to intravenous glucose is not delayed as it is following oral glucose and the insulinogetic index throughout the test is similar to that of non-diabetics.

It is difficult to explain the discrepancies in the tolerance to oral glucose in patients with otherwise similar tolerance to intravenous glucose. It may be that the IVGTT is a less valid test of glucose intolerance. The beta-cell may retain a normal responsiveness to intravenous glucose, tolbutamide and amino acids, irrespective of its sensitivity to oral glucose. There is no detectable anomaly of tissue insulin sensitivity. GH responses are frequently aberrant: they are either absent during hypoglycaemia and hyperaminoacidemia or paradoxical during hyperglycaemia; in this latter instance, they do not correlate with the degree of glucose intolerance.

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