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Abnormal Serum Growth Hormone Responses in Genetically Potential-Diabetic Male Patients with Normal Oral Glucose Tolerance: Evidence for an Insulin-Like Action of Growth Hormone in Vivo

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Summary. Serum growth hormone, immunoreactive insulin, plasma fatty free acids and blood sugar were measured during oral glucose, cortisone primed oral glucose and intravenous glucose tolerance tets and during intravenous tolbutamide test in 25 normal and 24 potential diabetic (offspring of two diabetic parents) males, closely matched for weight and age. Only potential diabetics with normal blood sugar levels during the oral, cortisone-primed and intravenous glucose tolerance tests were selected for study. — Mean serum growth hormone concentrations were significantly higher in the potential diabetic group at one or more intervals in each of the tests. The potential diabetic group showed a paradoxical rise in growth hormone during the first 60 min of the oral glucose tolerance test and to a less marked degree in the cortisone-primed oral glucose tolerance test. — Serum insulin was significantly reduced in these potential diabetics (who had been selected for their normal carbohydrate tolerance) during the oral, cortisone-primed and intravenous glucose tolerance test but not during the tolbutamide test. The presence of normal blood sugar responses and reduced insulin levels suggested increased sensitivity to endogenous insulin in the potential diabetic group, despite elevations in serum growth hormone. — Abnormal growth hormone responses occurred in 50% of the potential-diabetic group and an abnormal response in one test was usually associated with abnormal responses in each of the other types of tests. — When the potential-diabetics were subdivided into those with ('responders') and those without ('non-responders') an abnormal growth hormone response, it was the 'responders' who as a group showed increased sensitivity to endogenous insulin. Thus the abnormal growth hormone responses observed appeared to be associated with acute insulin-like effects, rather than the more usual diabetogenic action of growth hormone.

Key words: Potential diabetes, glucose tolerance tests, insulin, growth hormone, free fatty acids, paradoxical growth hormone responses, cortisone premedication.

The diabetogenic effect of growth hormone in animals and in man has been studied in detail for many years [1, 2]. It remains unclear, however, if this hormone plays any part in the aetiology of idiopathic diabetes mellitus. Previous studies have suggested that fasting serum growth hormone may be elevated in 'prediabetes suspects' [3] and in some patients with minor abnormalities in glucose tolerance [4]. Earlier studies from this laboratory have reported the serum growth hormone responses of non-obese male potential-diabetics (offspring of two diabetic parents) with no evidence of carbohydrate intolerance. The serum growth hormone response of these potential-diabetic males was greater than that of matched normal subjects during both an intravenous glucose tolerance test and following the intravenous administration of 1 g of tolbutamide [5]. Preliminary observations have also suggested an abnormal serum growth hormone response in potential-diabetic males during the oral glucose tolerance test [6]. The results suggested that an abnormality of growth hormone secretion may be present in potential-diabetic patients prior to the development of carbohydrate intolerance.

These observations prompted further studies on growth hormone secretion in this group of patients (a) in order to extend the observations to a larger group, (b) to ascertain the incidence of 'abnormal responses', (c) to determine if an 'abnormal response' occurred consistently in a given individual when challenged with several different stimuli, and (d) to attempt to ascertain the significance of the abnormal response in terms of the other parameters measured during the various tests.

Materials and Methods

Potential-Diabetic Subjects

The patients studied were non-obese (less than 120% of ideal weight[1]) male offspring of parents both of whom had well documented idiopathic diabetes mellitus. They were all within the age range of 16—45 years. All had been tested with oral glucose (OGTT), intravenous glucose (IVGTT) and cortisone-primed

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1 Metropolitan Life Insurance Company Tables, 1959.

oral glucose tolerance (COGTT) tests and none of the tests resulted in an abnormal blood sugar response. For the IVGTT an 'abnormal' test was defined as one in which the Kg value was less than 1.2% per minute. In the OGTT and COGTT an 'abnormal' test was defined as a test in which one or more blood sugar concentrations exceeded a limit determined by the mean plus 2 standard deviations of values obtained in a sex-age matched group of normal subjects with no family history of diabetes, all of whom were within 20% of their ideal weight [7].

Normal Subjects

A similar number of non-obese males with no family history of diabetes mellitus were tested in a manner identical to the potential-diabetic patients.

Tests

Before each test all individuals were instructed to consume a diet containing at least 250 g of carbohydrate per day for three days. The tests were all performed in the morning following an overnight fast. Patients arrived in the laboratory by their usual method of transportation; they rested for 15—30 min before an indwelling needle was placed in an antecubital vein and the fasting sample obtained.

The OGTT employed a dose of 100 g. The COGTT was according to Fajans and Conn [8]. For the IVGTT a dose of 0.5 g glucose/kg body weight was injected over a 2—4 min period. The intravenous tolbutamide test (IVTT) consisted of 1 g tolbutamide (courtesy of the Upjohn Company, Kalamazoo, Michigan) injected intravenously in a volume of 20 ml over 3 min [5]. Samples were taken for the determination of blood sugar (BS; 9), plasma free fatty acids (FFA; 10), serum insulin (IRI; 11) and growth hormone (GH; 12). The insulin values reported were obtained by reference to human insulin standards (courtesy of Dr. M. Root, Eli Lilly Co.) and the growth hormone concentration by reference to NIH-Wilhelmi growth hormone preparation 'NIH-GH-HS 968C' (courtesy of Dr. A. Wilhelmi).

A quality control serum was run with each assay and it measured 72.9 ± 0.8 μU/ml IRI (mean ± standard error of mean; $n = 69$) and 3.01 ± 0.16 ng/ml GH ($n = 32$). The lower limit of sensitivity in the growth hormone assay was 0.25 ng/ml of serum; all lesser values were designated zero.

Basic statistical analysis was performed by conventional techniques [13]. A correlation matrix was computed for all the substrate-hormone results, for the demographic characteristics (age, height, weight and percentage of 'ideal' weight) and for data derived from the various tests (eg: area under the IRI curve, regression coefficient of linear regression of IRI on BS, ratio of BS area in time interval 0—60 min/BS area in time interval 0—300 min — OGTT etc.). The unpaired 't' test was used to detect significant differences between the group.

Results

The basic statistical analysis showed that many of the hormone and substrate concentrations were not normally distributed. It appeared that log transformation of these data removed most of the skewness and kurtosis [14]. Statistical comparisons were therefore made and correlation coefficients computed before and after log transformation of the data: since many of the GH concentrations were recorded as zero, it was not possible to use the log transformation in the analysis of the GH data. The groups were closely matched for age, height, weight and percentage 'ideal' weight (Table 1).

Table 1. *Comparison of age, height, weight and percentage ideal weight of the two basic study groups*

	Normals ($n = 26$)	Potential diabetics ($n = 24$)	p
Age (yrs)	27.0 ± 5.3[a]	26.9 ± 11.1	n.s.
Height (cm)	182.1 ± 7.4	176.8 ± 12.2	n.s.
Weight (kg)	77.9 ± 8.3	71.6 ± 16.0	n.s.
% Ideal weight	103.5 ± 7.2	101.7 ± 7.7	n.s.

[a] Standard Deviation

a) *Oral Glucose Tolerance Test (25 Normal and 24 Potential-Diabetic Subjects)*

The results of the OGTT are summarized in Fig. 1. Although the mean blood sugar values of the potential-diabetic patients were higher than normal at each time interval, the difference between the groups was never significant, nor was the difference between the areas under the blood sugar curves. The mean IRI values in the potential-diabetic group were lower than normal in the first three hours of the test and the difference was significant at the 15 min point ($p < 0.05$). The mean fasting GH was higher in the potential diabetics than the normals and there was an anomalous rise in GH in this group during the first hour of the test. The difference in mean serum GH between the groups was significant at the 15, 30, 45, 60 and 90 min points ($p < 0.05$). The rise in GH concentration at the 4 and 5 h points was similar in both groups. Fasting FFA were similar, but the decline following the glucose ingestion was slightly less marked in the potential-diabetic group. The difference between the mean values at 90 min was significant following log transformation of the data ($p < 0.05$) (the numerical distribution of the FFA values at 90 min was significantly skewed and kurtosed but was normalized by log transformation). The rebound in FFA was similar in both groups. Although it is not apparent from Fig. 1, computation of the derived data from the tests revealed that the peak IRI occurred, on average, slightly later in the potential-diabetic group than in the normal (78.1 vs 67.8 min), although this difference was not statistically significant. There was in both groups a significant

correlation between the serum GH concentrations at 30, 45 and 60 min and the time at which peak IRI occurred ($r=0.701$ and $r=0.810$ for potential diabetics and normals respectively, $p<0.01$). There was an inverse correlation between the serum GH concentration in the 30 to 60 min period and the proportion of total insulin secretion that occurred in the first 60 min (area under IRI curve 0—60 min/area under IRI curve 0—300 min; $r=0.461$ and $r=0.464$, $p<0.05$ for potential diabetic and normal groups respectively). The serum GH in the 30 to 60 min period also correlated directly ($p<0.05$) in both groups with the concentrations of IRI and BS at the four and five hour time intervals. In contrast to these findings related to the early serum concentrations, the serum concentration of GH toward the end of the test (240 min) correlated directly (in both groups) with the IRI ratio ($r=0.450$ and 0.634 $p<0.05$ and <0.01 respectively for potential diabetics and normals) and with the FFA values at 240 min ($r=0.439$ and 0.454, $p<0.05$). Fasting blood sugar showed a significant rise with age in the potential diabetic group ($r=0.536$, $p<0.01$) but not in the normal group. There was also a direct correlation between age, weight and percentage 'ideal' weight and the early BS values (0—120 min) in the potential diabetic group, but not the normal.

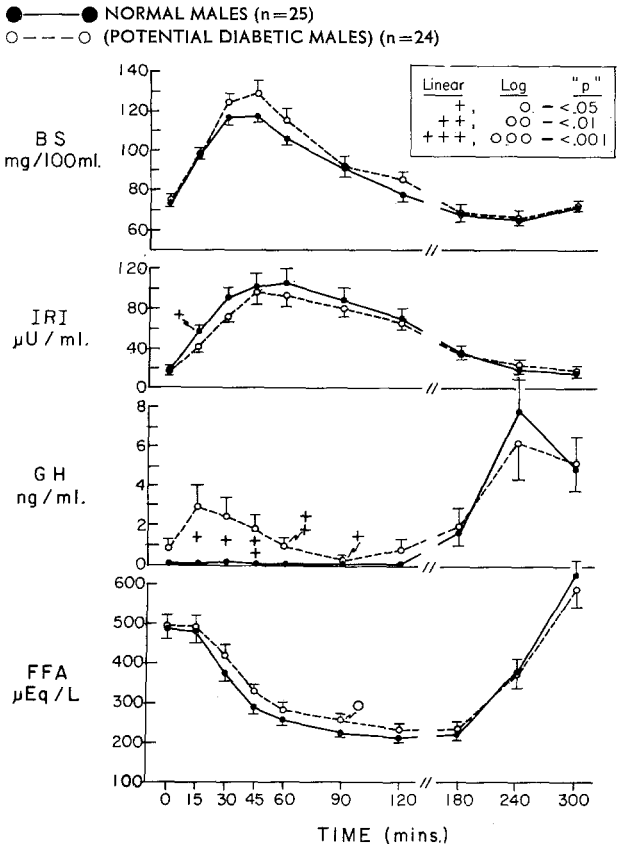

Fig. 1. Comparison of blood sugar (BS), serum immunoreactive insulin (IRI), serum growth hormone (GH), and plasma free fatty acids (FFA) between normal and potential diabetic male subjects during oral glucose tolerance tests

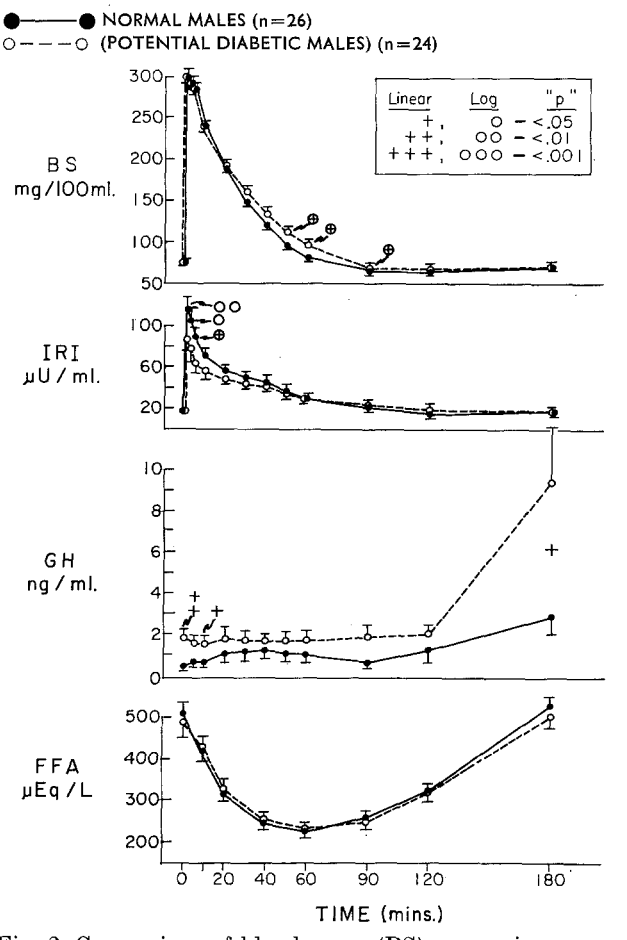

Fig. 2. Comparison of blood sugar (BS), serum immunoreactive insulin (IRI), serum growth (GH), and plasma free fatty acids (FFA) between normal and potential diabetic male subjects during intravenous glucose tolerance tests

b) *Intravenous Glucose Tolerance Tests (26 Normal and 24 Potential-Diabetic Subjects)*

The results of the IVGTT are summarised in Fig. 2. The peak BS was similar in both groups but the rate of fall of BS was slightly less rapid in the potential diabetics with the BS values at 50, 60 and 90 min being significantly higher in the potential diabetics than the normals ($p<0.05$). The difference in K_g only became significant following log transformation of the data (K_g rates 2.034 vs 2.383; $p<0.025$) which reduced but did not eliminate significant skewness and kurtosis.

The IRI concentrations in the early part of the test were lower in the potential-diabetic group, the difference being significant at 1, 3 and 5 min ($p<0.01$—

$p < 0.05$; again log transformation reduced but did not eliminate skewness and kurtosis). Fasting GH was higher in the potential diabetic group ($p < 0.01$) and remained higher throughout the test ($p < 0.05$ at 10 and 180 min) the rebound at the 180 min interval being more marked in the potential diabetics. There was a slight rise in GH in the normal group in the interval 0 to 40 min, falling to a nadir at 90 min before rising again at 180 min. FFA concentrations were similar at all time intervals in both groups. From the correlation analysis it appeared that in the potential diabetic group, the early rise in BS (1—5 min) was inversely related to the fasting GH ($r = 0.551$, $p < 0.01$). In the normal subjects, the magnitude of the early serum GH levels (10 to 60 min) was related to the rapidity with which the BS fell in the early part of the test (20 to 60 min) and thus with the K_g value ($r = -0.684$, $p < 0.01$). On the other hand, in the potential diabetics it was the GH concentration that occurred in the later part of the test (90 and 120 min) that correlated with the rate of fall of BS during the first 60 min of the test ($r = 0.523$, $p < 0.01$); this correlation was not seen in the normal group. In both groups, the K_g value and the regression coefficient (IRI upon BS) were directly related to the IRI area ratio (0—10/0—60 min; $p < 0.01$) and this ratio was directly related to the rise in GH in the early part of the test (10—90 min; $p < 0.01$) in the normals and later (60—120 min; $p < 0.05$) in the potential diabetics. There was a tendency for GH to be higher at all time intervals in the younger potential diabetics ($p < 0.01$—0.05).

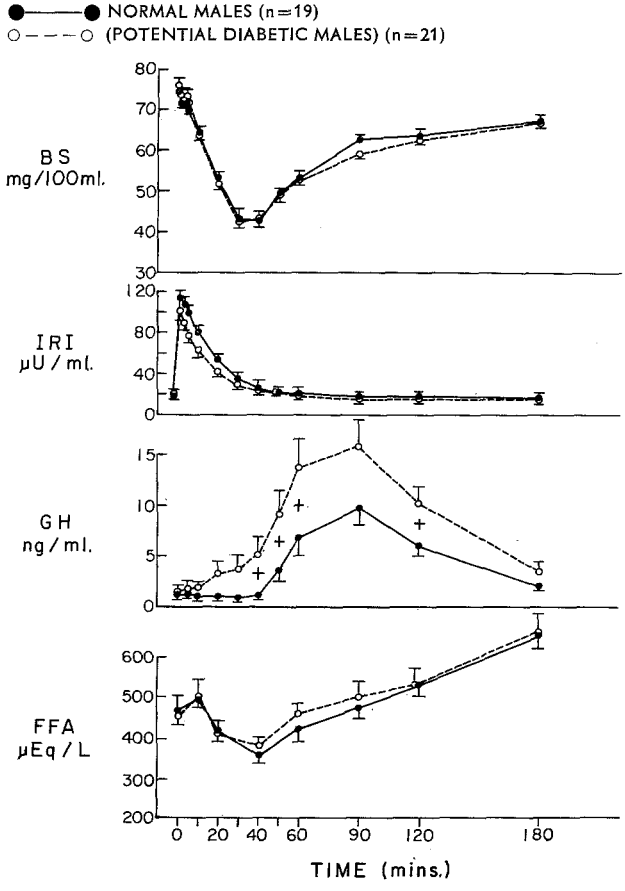

Fig. 3. Comparison of blood sugar (BS), serum immunoreactive insulin (IRI), serum growth hormone (GH), and plasma free fatty acids (FFA) between normal and potential diabetic male subjects during intravenous tolbutamide tests

The area under the IRI curve (0—10 and 0—60 min), the IRI area ratio (0—10/0—60 min), the peak IRI, the regression coefficient (IRI upon BS), and the BS area ratio (0—10/0—60 min) were all significantly lower in the potential-diabetic group compared to the normals ($p < 0.05$—$p < 0.01$).

c) *Intravenous Tolbutamide Tests (19 Normal and 21 Potential-Diabetic Subjects)*

The results of the IVTT are summarised in Fig. 3. The rate and magnitude of the fall in BS concentration following tolbutamide was similar in both groups. The return of BS concentration towards normal was slightly faster in the normal subjects than in the potential diabetics, but there was no significant difference between the groups. The rise in IRI following the administration of tolbutamide was smaller in the potential-diabetic patients, but the difference between groups was not significant at any time interval. Fasting GH was similar in both groups; in the normal group the GH concentration remained constant for the first 40 min and then rose to a peak of 9.8 ng/ml at 90 min (60 min after the BS had reached its nadir). In the potential-diabetic group however, there was a rise in GH in the 5 to 40 min period by which time there was a significant difference between the groups ($p < 0.05$); GH continued to rise, the peak occurred at 90 min (15.8 ng/ml). The GH concentration was higher in the potential diabetics at all time intervals and the differences were statistically significant at 40, 50, 60 and 120 min ($p < 0.05$).

Fasting FFA were similar in both groups and showed a similar rise at 10 min (due to the measurement of tolbutamide in the Dole procedure; [15]). FFA fell to a nadir at 40 min, and then rose to values in excess of fasting at 180 min. The magnitude of fall in FFA was marginally less marked in the potential diabetics and the early rebound was a little quicker, although there were no significant differences between the means of the groups.

Correlation analysis showed that in the normal group, the magnitude of GH in the 50 to 90 min period was inversely related to the level of blood sugar in the 30 to 60 min period ($r = -0.470$ to -0.681, $p < 0.05$—0.01). There appeared to be a fairly constant relationship between BS and GH (BS 30 with GH 50 ($r = -0.47$), BS 30 and 40 with GH 60 ($r = -0.51$ and

-0.56), BS 50 and 60 with GH 90 min ($r = -0.68$ and -0.50), suggesting that the rise in GH was proportional to the drop in BS and that there was a delay factor of approximately 20 to 30 min. This inverse relationship between BS and GH was not seen in the potential diabetics; instead there was a direct relationship between the GH at 30, 40, 50 and 60 min with the BS at 40, 50, 60 and 120 min. This suggested that those potential diabetic patients who had an early rise in GH had a more rapid rebound in BS. In this relationship the delay period was approximately 10 min (GH 30 with BS 40, GH 40 with BS 50, GH 50 with BS 50 and 60 and 120 and GH 60 with BS 60 and 120 min ($r =$ 0.441 to 0.551, $p < 0.05$ to < 0.01).

Within the potential diabetic group there was also a strong correlation between the magnitude of the GH concentration fasting and 5, 10, 20 and 30 min after tolbutamide, with the FFA concentration at 90, 120 and 180 min ($r = 0.437$ to 0.759, $p < 0.05 - < 0.01$). This implied that the magnitude of the early GH concentration was related to the rebound in FFA concentration that occurred later in the test. Within the normal group there was a direct correlation between age ($p < 0.01$) and weight ($p < 0.01$) and the GH concentration fasting and through 90 min. Within the potential diabetic group, the peak IRI and all the IRI values, fasting through 60 min, were directly related to percentage 'ideal' weight ($r = 0.437$ to 0.624, $p <$ $0.05 - < 0.01$; there was no difference in percentage 'ideal' weight between the groups).

d) *Cortisone Primed Oral Glucose Tolerance Tests (25 Normal and 23 Potential-Diabetic Subjects)*

In this test (Fig. 4) the BS of the two groups were very similar, the normal group having marginally higher values during the first hour of the test (cf. OGTT, Fig. 1). Serum IRI concentration was lower in the potential diabetic patients from 30 to 180 min, and the difference was significant at 30 and 45 min ($p = < 0.05$). Fasting GH was marginally higher in the normal group than in the potential diabetics and there was a slight fall in the 60 min period following glucose ingestion. Thereafter the mean value rose to a peak of 4 ng/ml at 240 min which was approximately half that seen in the OGTT. The potential diabetic group again showed a slight anomalous rise in GH concentration in the first 60 min of the test (as in the OGTT) the difference between the groups being statistically significant at 45 min ($p < 0.05$). The early rise was, however, much less marked than in the OGTT. The late peak in the potential diabetic patients was greater than in the OGTT and occurred at 300 instead of 240 min. At this point the differences between the groups was highly significant ($p < 0.001$). In the COGTT the mean fasting FFA in the normal group was slightly higher than in the potential diabetics (cf Fig. 1) but thereafter the values in the two groups were similar.

Correlation analysis suggested that (as in the OGTT) the BS in the first two hours of the test were directly influenced by age in the potential diabetic but not in the normal group; and in this group the regression coefficient (IRI upon BS) was inversely correlated with age ($r = -0.579$, $p < 0.01$). The early GH concentrations (fasting through 60 min) were related directly to age in the normal group. There was a

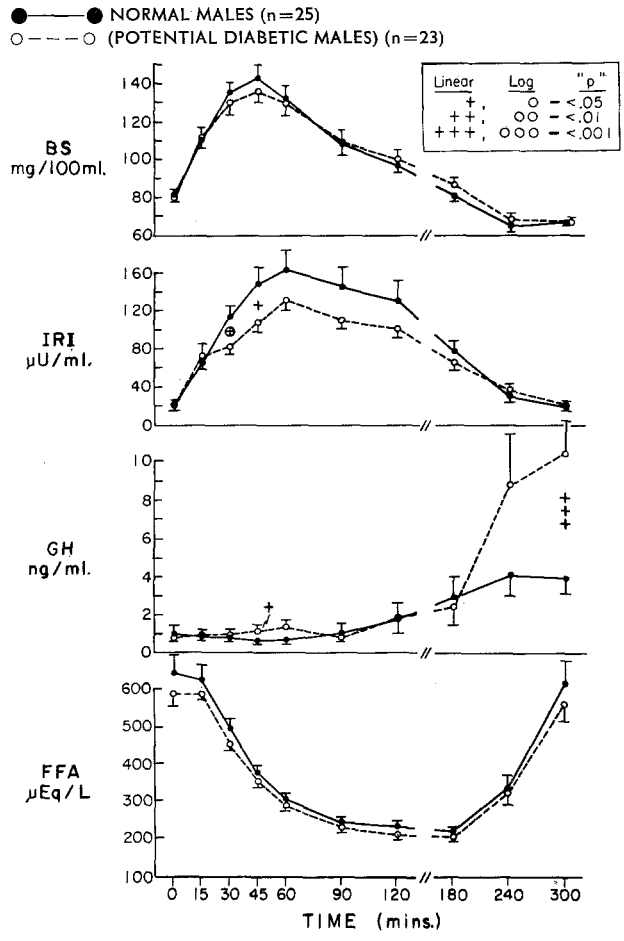

Fig. 4. Comparison of blood sugar (BS), serum immunoreactive insulin (IRI), serum growth hormone (GH), and plasma free fatty acids (FFA) between normal and potential diabetic male subjects during cortisone-primed oral glucose tolerance tests

significant correlation in both groups between the GH at 30 and 45 min period and the BS at 300 min.

The cortisone premedication for the COGTT (100 mg in those less than 160 lbs and 125 mg in those greater than 160 lbs taken in divided doses, one 8 h and the other 2.5 h before the test) resulted in a more marked increase in the fasting BS over that of OGTT in the normal subjects than in the potential diabetics (8.92 vs 3.64 mg per 100 ml; $p < 0.001$) (Table 2). This was complementary to the observation that the potential diabetics, who had higher BS during the OGTT, had lower mean BS values (than the normal group) during the first hour of the COGTT. Despite the 8.9 mg per 100 ml rise in fasting BS in the normal group

Table 2. *Change in fasting concentrations of blood sugar, serum insulin, plasma free fatty acids and growth hormone following premedication for cortisone-primed oral glucose tolerance tests in normal and potential diabetic males aged 16—45 years*

Parameter	Group[a]	Number	OGTT Mean ± SEM	COGTT Mean ± SEM	Δ	t[b]	p
Blood Sugar mg/100 ml	N	25	73.56± 1.43	82.48± 1.72	8.92	4.95	<0.001
	P	22	76.14± 1.61	79.77± 1.90	3.64	2.189	<0.025
Serum Insulin μU/ml	N	25	18.56± 2.08	19.04± 1.63	0.48	0.282	NS
	P	22	18.68± 1.94	21.00± 1.87	2.32	1.011	NS
Plasma Free Fatty Acids μEq/L	N	25	486.8±26.7	648.8 ±45.5	162.0	4.145	<0.001
	P	19	482.6 ±26.1	594.2±39.4	111.6	2.916	<0.005
Serum Growth Hormone ng/ml	N	24	<0.25± 0	0.96± 0.38	0.96	2.535	<0.01
	P	22	0.91± 0.58	0.78± 0.11	−0.13	0.225	NS

[a] Normal = N, Potential Diabetic = P OGTT = Oral glucose tolerance test COGTT = Cortisone-primed OGTT
[b] Paired 't' test Δ = difference between OGTT and COGTT

Table 3. *Number of male subjects with two or more elevated serum GH values during a test*

Group		OGTT	IVGTT	IVTT	COGTT
Controls	Abnormal GH	2 (8%)	2 (8%)	2 (11%)	3 (12%)
	Total	25	26	19	25
Potential Diabetics	Abnormal GH	12 (50%)	10 (42%)	11 (52%)	11 (48%)
	Total	24	24	21	23
	"p"[a]	<0.01	<0.02	<0.02	<0.02

[a] Difference in proportion of abnormal tests in the two study groups (by Chi Square analysis)

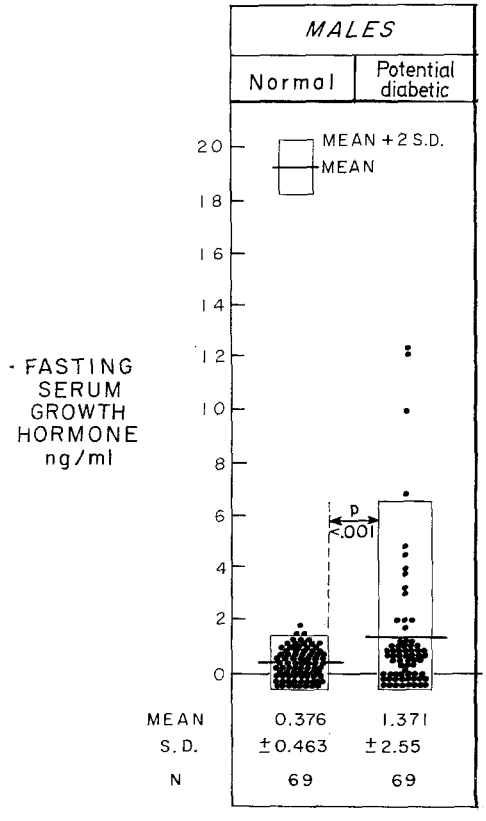

Fig. 5. Comparison of the fasting levels of serum growth hormone in 26 normal and 24 potential diabetic male subjects. In each subject, the three values obtained from the fasting sample of the IVGTT, OGTT and IVTT are depicted

($p<0.001$), there was no significant rise in fasting IRI. The potential diabetics showed a 3.6 mg per 100 ml rise in BS ($p<0.025$) and a 2.3 μU/ml rise in fasting IRI (ns). Fasting FFA also showed a greater rise in the normals than in the potential diabetic (162 vs 111.6 μEq per L; ns). The mean fasting GH was significantly higher following cortisone pre-treatment in the normal group, but fell after cortisone pretreatment in the potential diabetic patients. It was of interest that the cortisone pretreatment increased fasting GH in the normals while reducing the 240 min peak level whereas in the potential diabetics it appeared to suppress fasting GH (and the early anomalous rise) while accentuating the 240 and 300 min values.

Cortisone pretreatment as compared with OGTT shortened the interval between glucose administration and the peak IRI concentration in both groups. In the normal subjects, the time to peak insulin fell from 67.8 to 65.4 min (T = 2.4 min) while in the potential diabetics it fell from 78.1 to 70.4 min (T = 7.7 min).

In order to identify those individual tests that exhibited an 'abnormal' GH response, the mean GH plus two standard deviations were calculated for each time interval for the normal group in each type of test. Any test in which GH exceeded this limit at two or more time intervals was arbitrarily designated as having an 'abnormal' GH response (Table 3). The incidence of abnormal tests was higher in the potential diabetic group in each type of test (by Chi Square analysis ($p<0.02$).

Further analysis of the GH responses suggested that when a potential diabetic patient exhibited abnormal GH levels during one type of test it was likely

that the other types of tests would also show abnormal GH responses. Analysis of data from 25 normal subjects and 23 potential diabetics in whom results were available for the OGTT, IVGTT and IVTT indicated that the potential diabetic group had a significantly increased proportion of multiple abnormal tests (Table 4a). Data on all four tests were available from a smaller number of normal subjects ($n=18$) and potential diabetic patients ($n=20$) but Chi Square analysis again revealed an increased proportion of multiple abnormal tests in the potential diabetic group (Table 4b). There was only one member of the normal group who showed an abnormal test on more than one occasion.

Table 4a. *Abnormal serum growth hormone responses in subjects receiving three tests (OGTT, IVGTT, IVTT). Abnormal test — Serum GH at two or more time intervals exceeds mean plus 2 std. of normals*

Abnormal tests	Normals ($n = 25$)	Potential diabetics ($n = 23$)
0	19	8
1	5	4
2	1	6
3	0	5
	$p < 0.005$[a]	

[a] Difference in proportion of abnormal tests in the two study groups (by Chi Square analysis)

Table 4b. *Abnormal serum growth hormone responses in subjects receiving four tests (OGTT, IVGTT, IVTT, COGTT). Abnormal test — Serum GH at two or more time intervals exceeds mean plus 2 std. dev. of normals*

Abnormal Tests	Normals ($n = 18$)	Potential Diabetics ($n = 20$)
0	12	5
1	5	5
2	1	3
3	0	5
4	0	2
	$p < 0.05$[a]	

[a] Difference in proportion of abnormal tests in the two study groups (by Chi Square analysis)

Table 5. *Comparison between two subgroups of potential diabetic patients based on the presence or absence of an 'abnormal' growth hormone response*

	'Responders' $n = 14$	'Non-responders' $n = 10$	p
Age (yrs)	21.4	34.7	0.01
Weight (kg)	66.1	79.2	0.05
Height (cm)	176.0	177.8	NS
% Ideal weight	98.9	105.6	0.05
IVGTT: Insulin area ratio 0 — 10/0 — 60 min.	31.2	19.4	<0.02

e) *Fasting Serum Growth Hormone*

The mean fasting GH in the potential diabetic group was higher than that in the normals in each of three tests that were not preceded by cortisone premedication. In the IVGTT, the difference between the means was statistically significant ($p<0.01$). Fig. 5 summarizes the pooled data (from the IVGTT, OGTT, IVTT) for fasting GH in 26 normal subjects and 24 potential diabetic males. There was one observation in the normal group that exceeded Chauvenet's criteria for exclusion [16] and this value (6.5 ng/ml) was excluded when the mean ± 2 standard deviation limit was calculated. The pooled mean fasting GH in the potential diabetic group was significantly higher than normal by the unpaired 't' test (1.37 vs 0.38 ng/ml, $p<0.001$) irrespective of whether or not that one observation was excluded. This difference was also highly significant when tested by analysis of variance. There was also a significant difference between the groups when the mean of each individual's three fasting GH levels were compared between the two groups (1.52 vs 0.44 ng/ml; $p<0.02$).

f) *Responders versus Non-Responders*

In an attempt to clarify the significance of the 'abnormal' GH response, the potential diabetic group was subdivided into 'Responders' and 'Non-responders' on the basis of serum GH concentrations during the test. A 'responder' was defined as a subject who had two or more abnormal tests according to the GH criteria outlined previously. On this basis the 24 potential diabetics were subdivided into (a) fourteen '*responders*' (2 with 4 out of 4 abnormal tests, 5 with 3 out of 4 and 3 with 2 out of 4; 2 with 3 out of 3 and 2 with 2 out of 3) and (b) ten '*non-responders*' (5 with 0 out of 4 and 5 with 1 out of 4 abnormal tests).

The data on these two groups were then analysed separately and the difference between the means of each variable tested for significance by the unpaired 't' test.

The significant differences between the responders and non-responders are summarized in Table 5 and Fig. 6—9.

It can be seen that the 'responders' were significantly younger, lighter and thinner (as judged by 'percentage ideal weight') than the 'non-responders'. Of the 'derived' data (see Materials and Methods section), the only significant difference between the subgroups was in the insulin area ratios of the intravenous glucose tolerance test (Fig. 6), where it can be seen that the initial peak IRI concentration was lower in the 'non-responders' than in the 'responders'. There was in this group a second peak in IRI concentration occurring at 40 min. Mean serum IRI concentration was significantly higher in the 'non-responders' at all time intervals from 40 to 180 min ($p<0.05-<0.01$). This biphasic insulin secretory pattern was associated

with significantly higher blood sugar values over the period 20 to 50 min although the mean K_g values of the two subgroups were not significantly different. Fasting FFA concentration was higher in the 'non-responders' and the post glucose fall in FFAs was less marked than in the 'responders'. The mean FFA values for the subgroups were significantly different at 40 min ($p < 0.05$).

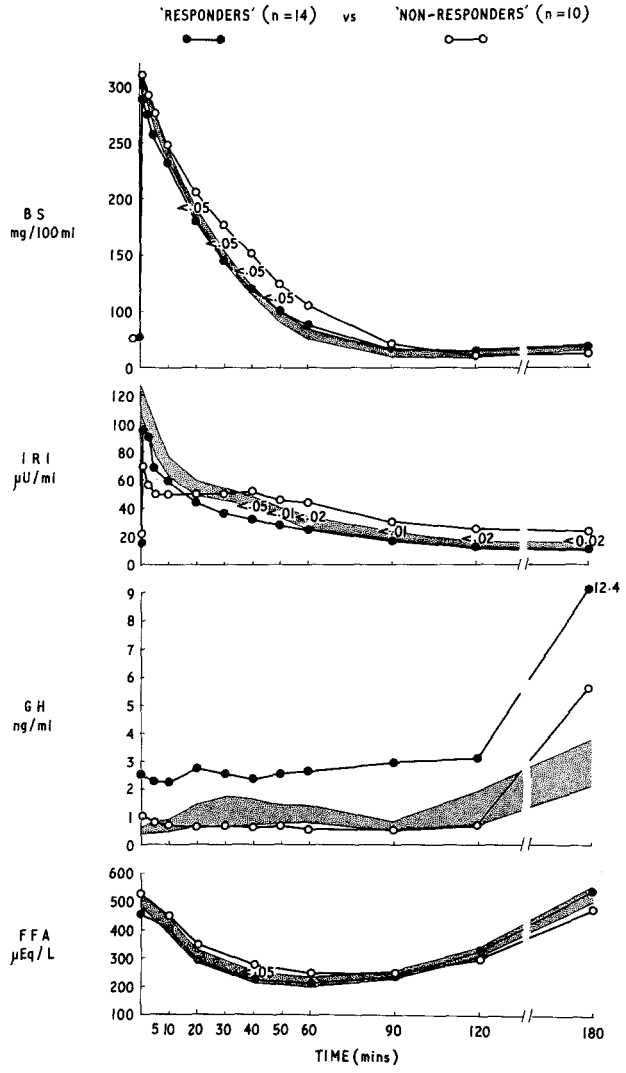

Fig. 6. Comparison of blood sugar (BS), serum immunoreactive insulin (IRI), serum growth homone (GH), and plasma free fatty acids (FFA) between 'responders' ($n = 14$) and 'non-responder' ($n = 10$) subgroups of the male potential diabetic patients during the intravenous glucose tolerance test. The mean $\pm$ 1 SEM of the normal group is shown in the shaded area. Mean values for the subgroups that were significantly different are indicated

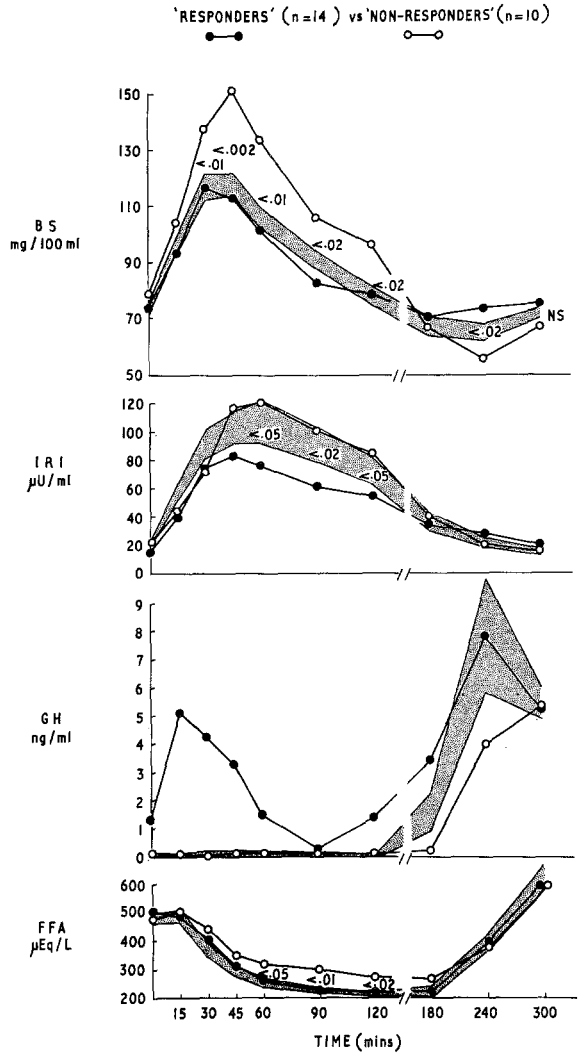

Fig. 7. Comparison of blood sugar (BS), serum immunoreactive insulin (IRI), serum growth hormone (GH), and plasma free fatty acids (FFA) between 'responder' ($n = 14$) and 'non-responder' ($n = 10$) subgroups of the male potential diabetic patients during the oral glucose tolerance test. The mean $\pm$ 1 SEM of the normal group is shown in the shaded area. Mean values for the subgroups that were significantly different are indicated

In the OGTT (Fig. 7) there was a marked difference in mean BS, IRI and FFA between the two subgroups. Mean fasting BS were similar, but all BS values between 30 and 120 min were significantly higher in the 'non-responders'. The reactive hypoglycaemia at 240 min was more marked in the 'non-responders'. Mean serum IRI was similar early and late in the test in both subgroups, but from 45 to 120 min the mean IRI values were lower in the 'responders'. Mean fasting FFA levels were similar in the subgroups, but the fall in FFA following the glucose load was significantly less marked in the 'non-responders'.

In the COGTT (Fig. 8) similar changes were seen, although the differences between the subgroups were less marked than in the OGTT. Although peak BS was significantly higher in the 'non-responders', there was

no exaggerated reactive hypoglycaemic phase. Mean FFA fell to significantly lower levels in the 'responders' but there was no significant differences between the IRI values.

The differences between the subgroups was least marked in the IVTT (Fig. 9) although the 'responders' had a more rapid and extensive fall in BS than the 'non-responders'. The nadir of BS was lower and 10 min earlier in the 'responders' and this subgroup exhibited a more rapid rebound in BS following the period of hypoglycaemia. These changes in BS occurred despite the fact that the mean IRI values for the 'responders' were lower than the 'non-responders' at all points throughout the test. There were no real differences in FFA values although the rebound in FFA (like the rebound in BS) was more marked in the 'responders' than in the 'non-responders'.

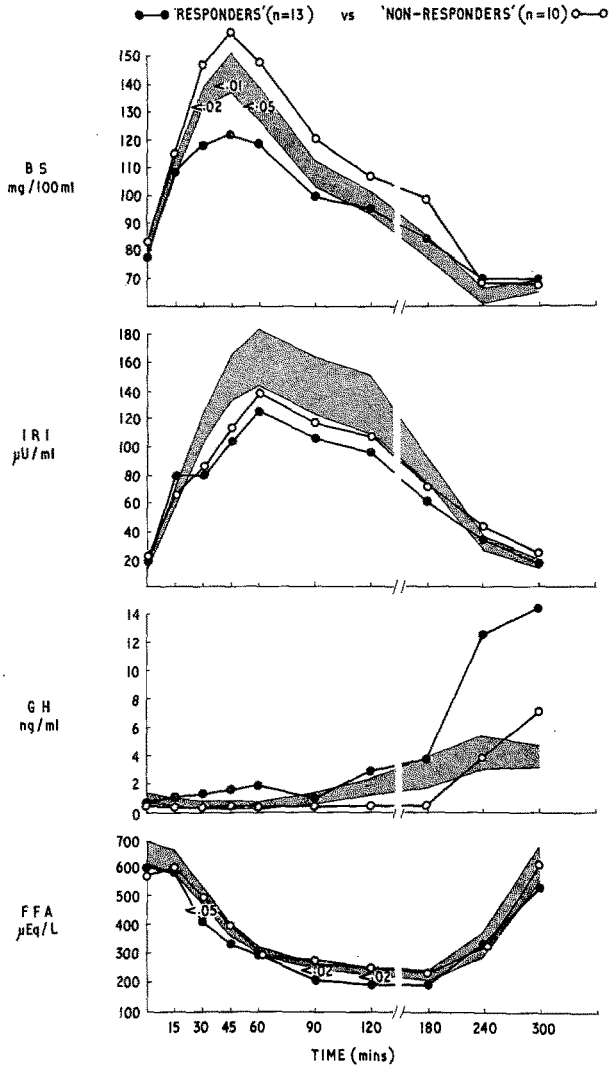

Fig. 8. Comparison of blood sugar (BS), serum immunoreactive insulin (IRI), serum growth hormone (GH) and plasma free fatty acids (FFA) between 'responder' ($n=13$) and 'non-responder' ($n=10$) subgroups of the male potential diabetic patients during the cortisone primed oral glucose tolerance test. The mean ± 1 SEM of the normal group is shown in the shaded area. Mean values for the subgroups that were significantly different are indicated

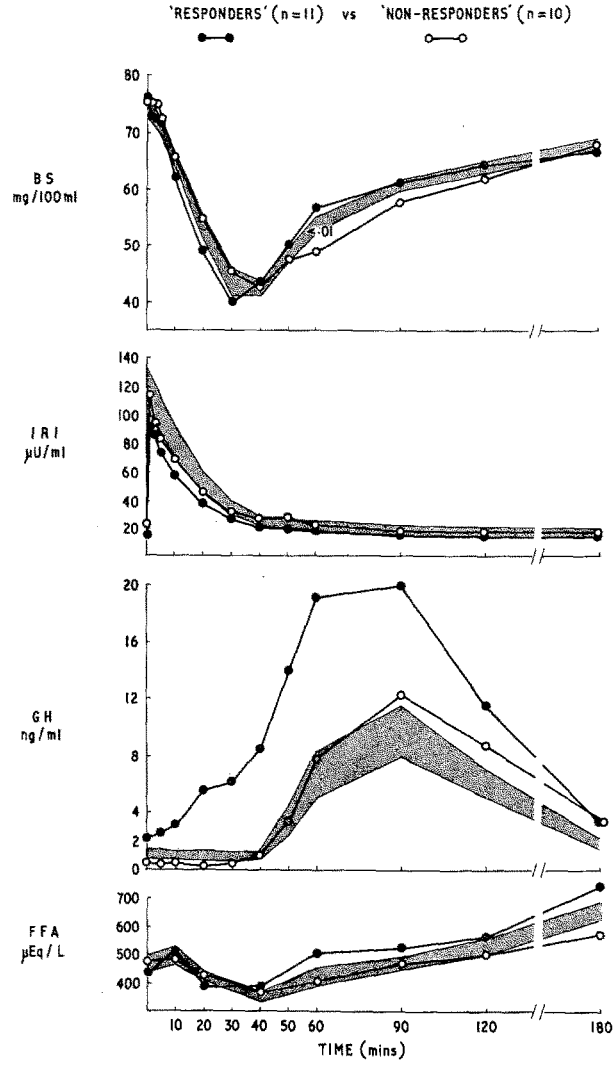

Fig. 9. Comparison of blood sugar (BS), serum immunoreactive insulin (IRI), serum growth hormone (GH) and plasma free fatty acids (FFA) between 'responder' ($n = 11$) and 'non-responder' ($n = 10$) subgroups of male potential diabetic patients during the intravenous tolbutamide test. The mean $\pm$ 1 SEM of the normal group is shown in the shaded area. Mean values for the subgroups that were significantly different are indicated

Discussion

Although growth hormone is known to be diabetogenic when administered to man [17] and animals [1], it is still unclear as to whether or not the hormone is involved in the aetiology of idiopathic diabetes mellitus. Elevated levels of GH have been noted in diabetes

with keto acidosis [18, 19] and in lactic acidosis associated with diabetes [19]. However, GH may be elevated in a variety of acute metabolic situations [19, 20, 21] although not usually to the levels seen in diabetic ketosis. The metabolic implication and alterations in plasma GH are not clear, although Yalow, Goldsmith and Berson [22] have shown that physiological GH responses that occur 4—5 h after oral glucose and after insulin induced hypoglycaemia may be causally related to the relative glucose intolerance that ensues, possibly by delaying the early insulin response to hyperglycaemia.

Controlled studies involving juvenile and maturity onset diabetic patients have not suggested a consistent abnormality in GH secretion [23, 24, 25]. An early rise in GH during the initial hyperglycaemic phase of the test has been reported in two of twelve diabetic patients [26]. Abnormal GH responses to oral glucose were also seen in a study of patients with minor abnormalities of glucose tolerance [4]; significantly elevated fasting GH concentration was also noted in non-obese males with mild glucose intolerance. An anomalous early rise in serum GH following oral glucose administration has also been reported in 'juvenile' diabetes [27], uraemia [28], acromegaly [29, 30], acute intermittent porphyria [31] and in active chronic hepatitis [32]. After intravenous administration of glucose, early growth hormone increases have been reported in Turner's syndrome [33], uraemia [34], the neonatal period [35] and in a patient with an optic glioma [28]. Although in this study the potential diabetic group showed a paradoxical rise in GH early after oral glucose there was no evidence of an early rise after intravenous glucose. In this test the difference between the groups was restricted to incomplete suppression of an elevated fasting GH concentration and a higher peak level at three hours in the potential diabetic group. The GH response to oral glucose in the potential diabetic group was biphasic and in this respect differed from the anomalous GH responses reported in the other studies. In each instance, when a paradoxical early rise was observed, GH fell from the early peak to a nadir of less than 1 ng/ml at 90—120 min before rising to a second peak (indistinguishable from normal) at 4—5 h. Cortisone pretreatment appeared to suppress incompletely the early GH rise in the potential diabetic group, while exaggerating the rise seen at 4 and 5 h. On the other hand, the late rise of GH in the normal group appeared to be attenuated in the COGTT (peak GH 7.9 and 4.1 ng/ml in OGTT and COGTT respectively). This biphasic GH response was rather similar to that shown to occur after insulin infusion in patients with uncontrolled diabetes mellitus [36].

It is difficult to determine the significance of the paradoxical early rise in GH seen in the OGTT which occurred in 14 out of 24 potential diabetic patients studied. There were only two of the 25 normal subjects who showed a significant rise in GH in the 0—90 min period (<0.25 to 0.8 and <0.25 to 3.8 ng/ml). The magnitude of early GH rise appeared to be related to the elapsed time between glucose administration and peak IRI concentration. Since a delayed rise in IRI is thought to be an early feature of diabetes mellitus [37], it is possible that this paradoxical rise in GH is also an early feature of the disease. It is impossible to determine whether this relationship is direct or indirect but it is of interest that cortisone pretreatment suppressed the early GH rise and was associated with a reduction in the time taken to reach the peak IRI concentration.

Cortisone pretreatment was also associated with a greater rise in fasting blood sugar and plasma FFA in the normal group compared to potential diabetics, and appeared to increase the fasting GH concentration in the normal group without influencing the value in the potential diabetics. These changes in fasting substrate and hormone concentrations suggest an alteration in basal energy metabolism induced by the cortisone medication and indicate that the response of the two groups is quite different. The cortisone premedication tended to reverse the difference between the groups (in terms of BS and plasma FFA) that were present in the OGTT.

In each of the tests, the potential diabetic patients showed evidence of increased sensitivity to endogenous insulin. The BS concentrations were similar to those of the normal group in the face of significantly diminished IRI response. This is difficult to reconcile with the results of Yalow *et al.* [22] and with the known diabetogenic effects of large amounts of exogenous GH [38] as well as the apparent resistance to endogenous insulin induced by the high GH levels in acromegaly [30, 39]. On the contrary, one might speculate that these small but significantly elevated GH responses observed in this study were compensating for the diminished insulin secretion. It has been shown in some circumstances, that growth hormone can have an acute insulin-like action [40, 41]. This speculation receives some circumstantial backing from the subgroup analysis based on the presence or absence of an 'abnormal' GH response.

This suggested that when the potential diabetic patients were subdivided on the basis of their serum GH responses, the subgroup with the consistently abnormal response ('responders') showed evidence of significantly better glucose tolerance, together with significantly greater inhibition of lipolysis in the presence of a diminished insulin response. These observations all suggest an increased sensitivity to endogenous insulin in the 'responders'. Luft and his co-worker have previously proposed an increased sensitivity to endogenous insulin in the patients that they classify as 'prediabetic' [42], since they have also observed normal glucose tolerance despite impaired insulin secretion. They subsequently tested the sensitivity of this group to exogenous insulin [43] and found this to be significantly increased, when compared with normal. The mechanism that accounts for this increased insulin

sensitivity is not clear but the results reported here allow the speculation that it may be based on an 'insulin-like' effect of growth hormone.

On the other hand, it is possible that the presence of a chronically diminished insulin response to elevations of blood glucose might alter the mechanisms governing growth hormone release by the pituitary, since it is known that the energy metabolism of the pituitary is insulin sensitive [44]. This suggestion would be in keeping with the GH responses to infused insulin in patients with established diabetes [36].

Despite efforts to match the normals and potential diabetics for age, weight and per cent ideal weight, there was a larger variation in these parameters in the potential diabetic group. The increased insulin sensitivity in the 'responders' may be related to their relative youth, although there was no statistical evidence for increased insulin sensitivity in the young control subjects. The relative youth of the 'responders' must be taken into consideration in assessing the significance of their abnormal growth hormone patterns. We do not know, however, of any evidence to suggest that the stress involved in taking part in these studies was more likely to produce abnormal growth hormone responses in the potential diabetics than the controls and do not consider this to be an adequate explanation for our observations.

A further possibility is that the paradoxical early rise in GH seen in the 'responders' was related to the more rapid and marked fall in plasma FFA in this subgroup, since plasma FFA concentrations have been shown to play a role in the regulation of GH secretion [45].

The presence in both groups of many correlations consistent with the known effects of GH are highly suggestive that this hormone plays an active role in blood glucose — insulin interrelationships and evidence for this has been published [46]. There is now little doubt that growth hormone levels are abnormally high in poorly controlled diabetics and that it is possible to lower these to near normal by intensive treatment with insulin [47, 48]. Although in other glucose tolerance test studies there has been little evidence for abnormalities in GH secretion in hereditary idiopathic diabetes mellitus, it is possible that the presence of a deranged glucose metabolism may obscure an abnormality of GH secretion that preceded in the development of overt diabetes. In keeping with this suggestion is the finding of abnormal GH responses to arginine infusions in a similar group of male potential diabetic patients with normal OGTT [49]; but no evidence of abnormal GH response in a comparable group of patients with abnormal COGTT.

It appears important to pursue these observations in potential diabetics in order to clarify the role of GH in human diabetes mellitus, although at present it is difficult to assess the patho-physiological significance of these findings.

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