Human Growth Hormone Secretion after Double Stimulation with Arginine in Normal and Insulin-dependent Diabetic Women

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SUMMARY

The standard intravenous arginine test was modified by infusing two equal doses of arginine eighty minutes apart. The serum growth hormone, blood glucose and plasma free fatty acid responses were studied in fifteen young juvenile-onset diabetic women and in seven healthy women of comparable age and weight. The biphasic growth hormone response to the two arginine infusions observed in normals did not occur in the diabetics. In addition, the diabetics differed from the normals in several qualitative aspects of growth hormone secretion. A derangement in the diabetic is postulated with impaired sensitivity or defective mechanism of growth hormone secretion. Diabetics with chronically elevated blood glucose levels had a blunted growth hormone response to arginine. In five patients with diabetic retinopathy, growth hormone response was somewhat blunted, probably due to higher blood glucose levels, as compared with findings in five patients without this complication. DIABETES *22*:694-705, September, 1973.

A possible relationship between disturbances of endogenous growth hormone (GH) secretion and the pathogenesis of diabetes and its complications has been suggested by several studies.[1-6] Among the various test procedures used to stimulate GH secretion, arginine has been credited with relatively few side effects compared to the hypoglycemic reactions which commonly follow intravenous insulin injections. A disadvantage of arginine is that frequently no GH response is observed following a single arginine infusion in subjects with normal pituitary function who readily respond to an arginine stimulus at a later date.[7-9] The effects of repeated intravenous infusion of arginine as a stimulus for growth hormone secretion in insulin-dependent adult diabetics have not been reported.

GH secretion was investigated in normal and diabetic women using a double arginine infusion technic in which a second infusion was administered eighty minutes after the initial thirty minute infusion. The purposes of this study were to determine whether aberrations of GH secretion were present in diabetic females and to evaluate the usefulness of the double arginine infusion test for assessment of GH secretion. Analyses were made to evaluate the effect of basal blood sugar upon the arginine-induced GH secretion and to ascertain if the GH responses correlated with retinopathy in diabetic patients.

MATERIALS AND METHODS

Fifteen women with juvenile-type diabetes (mean age 22.8 ± 1.5 years) were compared with seven normal women (mean age 23.7 ± 2.0 years). All but one of the diabetics were being treated with insulin.

The diabetics were divided in two groups: eight with long-term diabetes (duration greater than eight years; mean 13.1 ± 1.2 years) and seven with short-term diabetes from one to five years (mean 2.7 ± 0.6 years).

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Five of the long-term diabetics had retinopathy (four with numerous microaneurysms, one with proliferative retinopathy).

The normals had no personal or family history of diabetes and normal glucose tolerance as indicated by one or more oral tests. The mean per cent ideal weight was 103 ± 3.3 per cent for the normals and 99 ± 3.1 per cent for the diabetics (Metropolitan Life Insurance tables, 1959). None of the subjects was taking oral contraceptive medications.

All subjects were tested in the morning after an overnight fast. Insulin or tolbutamide treatment was withheld from the diabetics on the morning of the test. Seven diabetic patients were in the Hospital Teaching Unit of the Joslin Clinic for regulation of their diabetes. The subjects came from their hospital rooms or homes to the laboratory for the test. A needle was inserted in an antecubital vein and kept open with a slow infusion of isotonic saline solution. All subjects rested for forty minutes before the arginine infusion was started and blood samples were taken at time of arrival and every twenty minutes for four hours. Serum growth hormone,[10] blood glucose (by AutoAnalyzer)[11] and plasma free fatty acids[12] were determined at all time intervals in all subjects.

Initially 20 gm. of L-arginine monohydrochloride in a 10 per cent solution (courtesy of Dr. George Mouratoff, Cutter Laboratories, Berkeley, Calif.) was infused over thirty minutes. Eighty minutes after the start of the first infusion a second 20 gm. infusion of arginine was given. All subjects received the same absolute amount of arginine. Calculated per kilogram body weight, the administered individual arginine doses ranged between 0.29 and 0.42 gm. per kilogram (mean 0.35 ± 0.01 gm. per kilogram) in the normals and between 0.30 and 0.46 gm. per kilogram (mean 0.37 ± 0.01 gm. per kilogram) in the diabetics.

Correlations were calculated using conventional least squares methods (Pearson's).

RESULTS

Levels of growth hormone were fairly stable (changes not greater than 4.0 ng. per milliliter) in three normals and eight diabetics during the forty minute pre-infusion period. They rose in two normals and two diabetics and declined in two normals and five diabetics. Selecting an accurate value for baseline serum GH for further comparisons was difficult in view of the variations during the pre-infusion period. Therefore, the "baseline" was arbitrarily selected as the lowest value occurring between time zero and sixty minutes that was followed by a subsequently higher GH level. In this manner, it was possible to avoid "negative" growth hormone responses to arginine. In the normals this criteria resulted in the zero time interval level being selected in four, and the twenty minute level in the remaining three. In the patients with short-term diabetes, the baseline level was at zero minutes in three, twenty minutes in three and sixty minutes in one. In those with long-term diabetes, the "baseline" was at zero minutes in four, twenty minutes in two, forty minutes in one and sixty minutes in one. The mean baseline GH value was 8.6 ± 3.2 ng. per milliliter (± S.E.M.) in the normals, 5.0 ± 1.5 ng. per milliliter in patients with short-term diabetes and 4.0 ± 2.0 ng. per milliliter in those with long-term disease. These values were not significantly different.

Serum growth hormone response to double stimulation with arginine. A significant response was defined as an elevation in GH of 5 ng. per milliliter or more above the baseline level and/or a sustained level of GH above baseline when the arginine infusion occurred during a previous GH peak. All twenty-two subjects showed a serum GH response to at least one of the two arginine infusions; sixteen responded to both.

Five of the seven normals showed a response to both arginine infusions (table 1 and figure 1). One subject (T.S.) had no response to the first infusion but exhibited a marked response to the second. Another (A.B.) responded maximally to the first but the GH levels fell during the second infusion. Peaks occurred at forty or sixty minutes after the start of the first infusion and at forty, sixty or eighty minutes after the start of the second. The increase of GH from the baseline value to the peak value was expressed as the GH increment. This was 21.1 ± 7.3 ng. per milliliter after the first infusion and 30.3 ± 8.0 ng. per milliliter after the second. These were not significantly different. There was no significant difference between the mean peak values after the first infusion (mean 29.8 ± 8.7 ng. per milliliter) and that after the second (mean 37.0 ± 7.5 ng. per milliliter).

Compared to normals, the diabetics showed a less consistent response with a greater variety in secretion patterns (tables 2 and 3 and figure 1). Three of the fifteen diabetics showed no response to the first infusion but responded to the second (R.C., M.C. and M.S.). Two others responded only to the first (M.E. and C.S.). Only five of the diabetics showed the distinct two-peak response: two of the seven with short-term disease (B.I. and C.R.) and three of the eight with diabetes of

TABLE 1

Serum growth hormone (GH), blood glucose (BG) and free fatty acid (FFA) responses in normal females
(GH = ng./ml., BG = mg./100 ml., FFA = μEq./L.)
Arginine infused from 0 to 30 and 80 to 110 minutes

Subject		Time —40	—20	0	20	40	60
E.H.	GH	3.0	18.0	25.0	16.0	16.0	11.6
	BG	73	73	68	79	76	72
	FFA	630	590	680	490	290	330
A.B.	GH	6.6	21.0	25.0	38.0	64.0	64.0
	BG	85	89	101	100	85	84
	FFA	450	500	420	270	220	270
N.P.	GH	3.4	3.0	3.4	9.2	—	21.0
	BG	66	68	69	84	63	50
	FFA	350	400	360	270	140	130
T.S.	GH	1.0	1.2	—	0.8	1.6	1.4
	BG	61	61	—	59	49	44
	FFA	430	440	—	360	330	320
C.A.	GH	52.0	21.0	7.2	5.4	58.0	56.0
	BG	70	71	67	69	47	44
	FFA	490	460	510	220	150	170
G.B.	GH	9.6	5.6	5.6	9.6	25.0	32.0
	BG	76	71	76	93	75	59
	FFA	690	680	540	450	350	300
Q.M.	GH	10.2	7.6	4.2	4.4	16.0	16.0
	BG	74	70	69	72	64	60
	FFA	390	380	320	190	170	220
Mean	GH	12.2± 6.7	11.0± 3.2	11.7± 4.2	11.9± 4.7	30.1±10.2	28.8± 8.8
(± S.E.M.)	BG	72 ± 2.8	71 ± 3.2	75 ± 5.3	79 ± 5.3	65 ± 5.3	59 ± 5.6
	FFA	490 ±47	492 ±40	471 ±54	321±43	235±33	248±29

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long duration (L.J., K.M. and J.M.). In the five others the two responses fused into one peak, which was sustained for as long as eighty minutes in one patient (J.C.). Peak responses occurred between twenty minutes (i.e. during infusion) to eighty minutes after the start of either the first or the second arginine infusion and did not follow a consistent pattern.

In the patients with short-term diabetes the mean GH increment was 13.6 ± 8.3 ng. per milliliter after the first and 25.0 ± 8.5 ng. per milliliter after the second infusion. The mean peak responses were 18.7 ± 7.8 ng. per milliliter and 30.1 ± 7.3 ng. per milliliter, respectively. In the long-term diabetic group the mean GH increment was 27.0 ± 8.3 ng. per milliliter after the first and 26.4 ± 8.8 ng. per milliliter after the second infusion, and mean peak responses were 31.0 ± 7.4 ng. per milliliter and 29.7 ± 8.1 ng. per milliliter, respectively. In the short-term and long-term diabetic groups, there were no significant differences between the first and second responses when the mean GH increments or the mean peak responses were compared. Neither were there significant differences in the mean GH increments or peak responses between normals and diabetics or between the two diabetic groups.

Comparing the mean serum GH level of the normals to those of the diabetics with short- and long-term disease, the latter groups showed somewhat, but not significantly, lower peaks and the fall between the two peaks was not distinct (figure 1).

The mean of the total area under the curves was calculated for each group, but there were no statistically significant differences noted following group comparisons.

A significant negative correlation between baseline GH values and the GH increment to the second arginine infusion—but not to the first—was observed in the normals ($p < 0.05$) and patients with short-term diabetes ($p < 0.02$). In the long-term diabetic group there was no correlation between baseline GH and the GH increment of the first or second infusion.

Blood glucose (BG) response. Fasting BG (zero minutes) in the normals ranged from 67 to 101 (mean 75

TABLE 1 *(continued from page 696)*

Serum growth hormone (GH), blood glucose (BG) and free fatty acid (FFA) responses in normal females
(GH = ng./ml., BG = mg./100 ml., FFA = μEq./L.)
Arginine infused from 0 to 30 and 80 to 110 minutes

Subject		Time 80	100	120	140	160	180	200
E.H.	GH	5.4	4.0	21.0	32.0	38.0	13.2	3.6
	BG	70	75	71	68	76	74	78
	FFA	480	430	340	470	540	660	870
A.B.	GH	28.0	11.6	11.6	7.0	4.2	2.0	1.8
	BG	82	96	88	78	83	81	85
	FFA	450	320	270	330	680	840	930
N.P.	GH	13.6	13.6	25.0	18.0	13.6	8.0	8.2
	BG	57	61	56	50	55	71	70
	FFA	130	130	140	100	180	360	460
T.S.	GH	1.8	38.0	64.0	58.0	32.0	13.2	9.6
	BG	53	60	65	58	58	60	64
	FFA	330	350	290	290	310	440	550
C.A.	GH	13.2	7.0	28.0	64.0	64.0	58.0	25.0
	BG	55	62	59	53	59	64	61
	FFA	500	410	220	210	650	1020	1100
G.B.	GH	28.0	12.0	25.0	32.0	11.6	7.6	7.2
	BG	70	74	77	74	72	73	72
	FFA	430	440	330	370	680	850	860
Q.M.	GH	8.8	10.6	25.0	9.2	—	3.4	—
	BG	62	61	57	56	—	70	66
	FFA	280	230	230	280	360	380	410
Mean (± S.E.M.)	GH	14.1± 3.9	13.8± 4.2	28.5± 6.2	31.4± 8.5	27.2± 9.0	15.0± 7.3	9.2± 3.3
	BG	64 ± 3.9	69 ± 4.9	67 ± 4.4	62 ± 4.1	67 ± 4.6	70 ± 2.5	70 ± 3.1
	FFA	371 ±50	330 ±43	260 ±26	292 ±44	485 ±76	650 ±99	740 ±10

± 5.3) mg./100 ml. Arginine infusion resulted in a mean BG rise of 4 mg./100 ml. twenty minutes after the start of the infusion. This was followed by a mean fall of 20 mg./100 ml. from twenty to sixty minutes. The mean rise after the second infusion (from eighty to 100 minutes) was 5 mg./100 ml., which was followed by a mean fall of 7 mg./100 ml. By the end of the test BG levels were nearly back to baseline (table 1 and figure 1).

The patients with short-term diabetes (table 2 and figure 1) showed fasting BG between 90 and 338 (mean 194 ± 35) mg./100 ml. and generally exhibited the same pattern of BG response to arginine as the normal subjects, although BG peaks occurred later and the values were in the diabetic range. The mean peak BG rise following the first infusion was 26 mg./100 ml. After the peak, a mean fall of 17 mg./100 ml. was seen. The second infusion produced an average rise of 2 mg./100 ml. and was followed by a mean fall of 28 mg./100 ml. The BG levels were lower than the fasting at the end of the test.

Fasting BG levels in the long-term diabetic group ranged from 97 to 308 (mean 217 ± 25 mg./100 ml.) and were not significantly different from those of the subjects with short-term diabetes (table 3 and figure 1). The mean peak BG rise was 45 mg./100 ml. after the first infusion, which was followed by a mean fall of 5 mg./100 ml. The second average rise was 33 mg./100 ml. and the second mean fall was 12 mg./100 ml. It is apparent that BG levels in this group showed a tendency to rise throughout this test and were significantly higher (p ranging from < 0.05 to < 0.01) than in the short-term diabetic group at the 120 to 200 minute intervals.

Free fatty acid (FFA) response. Mean fasting FFA levels (figure 1 and tables 1 to 3) were lowest in normals and highest in the patients with long-term diabetes. Both arginine infusions caused a fall in FFA levels, which was most pronounced in the normals with a drop to 50 per cent of the pre-infusion level after the first infusion and to 70 per cent of the eighty minute level after the second infusion. The mean levels were significantly lower in normals than in the short-term diabetic group at twenty ($p < 0.02$), forty ($p <$

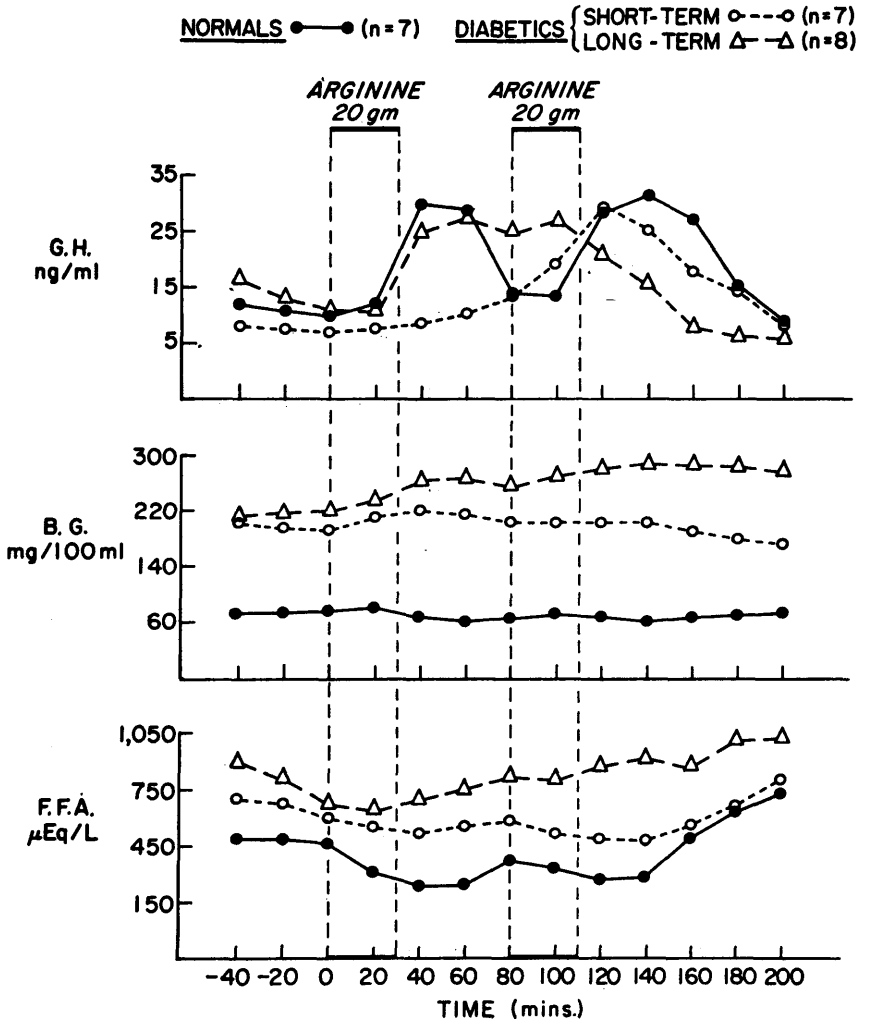

FIGURE 1

Mean levels of serum growth hormone (GH), blood glucose (BG) and plasma free fatty acid (FFA) in normal controls and in patients with short- and long-term diabetes. Significant differences detailed in text.

0.01), sixty ($p < 0.01$) and 120 minutes ($p < 0.05$). The FFA declined to 86 per cent and 81 per cent in the short-term diabetic group and only to 96 per cent and 98 per cent in the long-term diabetic group during the first and second infusions, respectively. Long-term diabetics had significantly ($p < 0.01$ to 0.001) higher mean FFA levels than normals at all time intervals except zero minutes and higher levels than short-term diabetics at 120 ($p < 0.05$), 140 ($p < 0.01$), 160 ($p < 0.01$) and 180 minutes ($p < 0.05$).

The fall in FFA levels in normals after the second infusion (260 μEq. per liter) was followed by a rebound rise of almost threefold (2.8 x) by 200 minutes (740 μEq. per liter). With short-term diabetes this rebound rise was 1.7-fold (482 to 811 μEq. per liter), whereas in the long-term group it was only 1.27-fold (812 to 1,031 μEq. per liter). In normals the rebound rise above both the initial fasting level and the nadir following the second arginine infusion correlated positively ($p < 0.05$) with the GH secretion expressed as the area under the total curve above baseline (0 to 200 minutes). This correlation did not occur in the diabetic groups.

Effect of blood glucose levels on growth hormone secretion. Fasting BG levels showed a significant positive correlation with baseline GH levels ($p < 0.01$) in normals but not in either of the diabetic groups. The fasting BG levels also correlated positively ($p < 0.05$) in normals with the peak GH response after the first arginine infusion and negatively ($p < 0.05$) with the GH increment and the peak GH response after the second infusion. Among the subjects with short-term disease fasting BG did not correlate with either the increment

TABLE 2

Serum growth hormone (GH), blood glucose (BG) and free fatty acid (FFA) responses in females with short-term diabetes
(GH = ng./ml., BG = mg./100 ml., FFA = μEq./L.)
Arginine infused from 0 to 30 and 80 to 110 minutes

Patient		Time —40	—20	0	20	40	60
R.C.	GH	10.2	12.4	12.4	13.2	12.0	8.8
	BG	382	350	338	350	356	366
	FFA	730	590	410	540	590	680
M.C.	GH	12.4	13.2	11.2	6.2	2.8	1.4
	BG	318	310	302	320	324	324
	FFA	810	720	590	540	700	730
J.C.	GH	10.2	3.6	2.4	1.4	1.6	25.0
	BG	112	111	109	125	149	140
	FFA	400	590	540	510	330	300
J.D.	GH	0.6	1.4	4.6	10.6	8.0	8.0
	BG	170	174	170	188	168	182
	FFA	590	500	500	470	450	490
B.I.	GH	4.6	11.6	9.6	6.6	11.6	12.4
	BG	185	185	184	207	239	233
	FFA	1480	1170	1080	990	830	820
C.R..	GH	9.2	8.0	7.6	13.2	21.0	10.6
	BG	170	172	166	200	204	184
	FFA	540	560	520	340	320	420
R.W.	GH	8.8	3.2	1.6	1.4	1.8	3.6
	BG	92	90	90	100	102	86
	FFA	390	560	620	550	460	410
Mean (± S.E.M.)	GH	8.0± 1.5	7.6± 1.8	7.0± 1.6	7.5± 1.8	8.4± 2.6	9.9± 2.8
	BG	204 ± 40	198 ±36	194 ±35	212 ±35	220 ±35	216 ±37
	FFA	705 ±141	670 ±87	608 ±82	562 ±76	525 ±72	550 ±73

(Continued on page 700)

or peak GH response. Fasting BG levels in the long-term diabetic group, however, showed a significantly *negative* correlation with the peak response after the first arginine infusion ($p < 0.02$) as well as with the increment above baseline following the second infusion ($p < 0.001$). In this group there was also a negative correlation ($p < 0.001$) between fasting BG and GH secretory response expressed as total area under the GH curve. No such correlation was present in either the normal or short-term diabetic group.

Patients, regardless of the duration of their diabetes, were divided in two groups according to fasting blood glucose level (figure 2). The first group (six patients) with fasting BG levels between 90 and 170 mg./100 ml. (mean 126 mg./100 ml.) showed a significantly greater response ($p < 0.01$), expressed as the GH area under the total curve, than the second group (nine patients) with fasting blood glucose levels ranging from 185 to 338 mg./100 ml. (mean 259 mg./100 ml.). Mean GH level in the low fasting BG group was significantly higher at 80 ($p < 0.05$), 100 ($p < 0.02$), 120 and 140 minutes ($p < 0.01$) when compared to the mean GH level of the high fasting BG group.

Arginine per kilogram body weight and growth hormone response. Since each subject received the same amount of arginine, the infused amount per kilogram body weight varied inversely with the weight of the individual (see above). In normals, GH response to the infusion, expressed as the area under the total curve, correlated positively ($p < 0.02$) with the amount of arginine per kilogram body weight. This correlation was not present in the two diabetic groups.

Patients with diabetic retinopathy. Five patients with diabetic retinopathy (mean age 24.4 years; mean duration of diabetes 14.6 years) were compared with five diabetics without retinopathy matched for age (mean age 24.0 years; mean duration of diabetes 8.2 years). The group with retinopathy showed a tendency toward blunted GH response and had significantly lower mean GH levels at the 120, 160 and 200 minute intervals ($p < 0.05$ to < 0.02) than the patients without retinopathy (figure 3). However, the fasting BG level of the retinopathy group was higher than that of the

TABLE 2 *(continued from page 699)*

Serum growth hormone (GH), blood glucose (BG) and free fatty acid (FFA) responses in females with short-term diabetes
(GH = ng./ml., BG = mg./100 ml., FFA = μEq./L.)
Arginine infused from 0 to 30 and 80 to 110 minutes

Patient		Time 80	100	120	140	160	180	200
R.C.	GH	3.8	2.4	13.6	13.2	11.6	13.2	11.6
	BG	346	344	330	346	338	318	306
	FFA	730	590	410	540	590	680	700
M.C.	GH	1.4	13.6	38.0	16.0	9.2	10.2	10.6
	BG	300	312	304	306	278	272	272
	FFA	710	670	710	660	750	850	910
J.C.	GH	64.0	64.0	64.0	64.0	64.0	58.0	25.0
	BG	136	136	136	125	120	118	110
	FFA	260	250	260	260	310	360	520
J.D.	GH	12.0	28.0	44.0	32.0	12.4	7.0	4.0
	BG	168	174	168	168	156	144	142
	FFA	360	310	300	350	440	520	790
B.I.	GH	4.6	3.0	4.6	10.2	7.6	5.0	3.2
	BG	221	204	222	219	195	184	185
	FFA	1190	1040	900	780	920	960	1000
C.R.	GH	5.6	3.8	13.6	16.0	10.6	3.4	2.4
	BG	174	184	198	192	178	164	156
	FFA	610	440	370	380	510	650	730
R.W.	GH	7.2	18.0	25.0	21.0	11.6	7.0	3.2
	BG	82	82	78	76	70	66	70
	FFA	320	270	270	300	380	570	760
Mean	GH	14.0± 8.4	18.9± 8.3	28.9± 7.8	24.6± 7.0	18.1± 7.6	14.8± 7.2	8.5± 3.0
(± S.E.M.)	BG	203 ±34	205 ±35	205 ±33	204 ±36	190 ±34	180 ±33	177 ±32
	FFA	592 ±12	525 ±11	504 ±99	482 ±78	574 ±84	660 ±76	811 ±62

group without retinopathy, which may have accounted for the lower GH response.

DISCUSSION

Because false negative results with the single arginine infusion test are relatively frequent in individuals who respond positively upon repeated testing, the double arginine test appears to be a logical approach to avoid the need for retesting. The present study has demonstrated the usefulness of the double arginine infusion in females in securing a significant GH response in every subject. All subjects in this study showed a response to at least one of the arginine infusions. There were wide variations in individual GH responses but a delay or attenuation of the second response by the previous stimulation, as reported by others,[13] was not noted.

In this study, no significant differences in quantitative GH responses could be observed between young female insulin-dependent diabetics and normal women of comparable age. Similarly no quantitative difference was noted between diabetics of short and long duration.

Attempts have been made in the past to investigate GH secretion during various stages of diabetes. Earlier studies from this laboratory have shown significantly higher than normal GH responses in prediabetic males (offspring of two diabetic parents) after intravenous administration of tolbutamide or glucose[14] and after oral glucose tolerance tests.[15] In addition, it has been shown by Knopf et al. that male relatives of diabetics without carbohydrate intolerance show greater serum GH responses during arginine infusions than do healthy control subjects.[16] Sabeh et al.[17] reported lower peak GH levels associated with newly discovered glucose intolerance. Children with newly diagnosed diabetes were reported to have normal GH rises after arginine and insulin stimuli in one study[18] while others found greater rises after arginine in a similar group of children.[19] The latter study showed no difference in arginine stimulated GH levels between children with diabetes of longer duration and normal children. This was also confirmed by Baker et al.[20] Johansen et al.[21] reported higher mean GH secretion in juvenile diabetics when monitoring GH levels over twenty-four hours. Hansen[22] also found an abnormal rise in GH during muscular

TABLE 3

Serum growth hormone (GH), blood glucose (BG) and free fatty acid (FFA) responses in females with long-term diabetes
(GH = ng./ml., BG = mg./100 ml., FFA = μEq./L.)
Arginine infused from 0 to 30 and 80 to 110 minutes

Patient		Time —40	—20	0	20	40	60
M.D.	GH	2.2	1.0	0.8	4.0	44.0	58.0
	BG	129	129	128	152	179	187
	FFA	1020	760	660	560	560	570
L.J.	GH	1.2	1.0	0.8	12.4	13.6	8.4
	BG	189	219	219	255	276	285
	FFA	980	980	770	760	870	930
M.S.	GH	16.0	16.0	44.0	21.0	13.6	10.2
	BG	202	208	207	236	242	254
	FFA	810	640	590	660	760	770
K.M.	GH	1.0	1.0	0.8	11.2	64.0	64.0
	BG	84	91	97	144	204	208
	FFA	1070	1050	610	390	460	470
J.M.	GH	7.4	1.4	1.2	1.0	9.2	25.0
	BG	242	250	250	266	294	286
	FFA	1410	1180	1020	940	980	1170
M.E.	GH	64.0	52.0	38.0	32.0	16.0	21.0
	BG	310	304	286	284	300	270
	FFA	250	280	240	300	340	350
L.C.	GH	3.0	4.4	1.8	1.4	13.6	21.0
	BG	240	228	244	258	280	290
	FFA	590	740	760	730	720	710
C.S.	GH	38.0	25.0	1.2	7.6	25.0	16.0
	BG	318	318	308	308	326	318
	FFA	1070	970	790	880	980	1040
Mean (± S.E.M.)	GH	16.6± 8.0	12.7± 6.4	11.0± 6.5	11.3± 3.7	24.8± 6.8	27.9± 7.4
	BG	214 ± 28	218 ± 27	217 ±25	237 ±21	262 ±17	262 ± 15
	FFA	900 ±124	825 ±100	680 ±78	652 ±79	708 ±84	751 ±100

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exercise associated with poorly controlled early diabetes, which was ameliorated by careful blood sugar regulation. Burday et al.[23] found the peak GH response after arginine and insulin lower in patients with maturity-onset diabetes than in insulin-dependent patients and normals. The latter two groups showed equal responses. Merimee et al.[24] reported no GH response to a single arginine stimulation in five patients with maturity-onset diabetes.

Although no gross quantitative differences are shown in this present study, the diabetics differed from the normals in several qualitative aspects of GH secretion, which suggests an impaired sensitivity to stimulators of GH release and/or a defect in the GH release mechanism. The responses in the diabetics were less consistent, showed a wider range in amplitude and varied more from one infusion to the other when compared to normals. This is consistent with the observation of Burday et al.[23] Lundbaek et al. found the twenty-four hour GH secretion in juvenile diabetics "wildly fluctuating."[25]

In the normals, some interrelationships among fasting BG levels, GH baseline levels and GH responses to repeated stimulations as well as between GH secretion and FFA levels were found. GH baseline levels were related to fasting BG in normals. This is remarkable because fasting BG levels differed only slightly in these subjects. In both diabetic groups, GH baselines did not correlate with fasting BG levels, although differences in BG levels among patients were marked. Recently, a positive correlation of fasting GH and BG was shown in a group of 315 diabetics.[26] Undoubtedly, large numbers of patients facilitate identification of relationships.

Baseline GH levels in normals seemed to have a delayed effect on GH response, which became manifest only during the second stimulation; those with higher baselines show a lower GH increment after the second

TABLE 3 *(continued from page 701)*

Serum growth hormone (GH), blood glucose (BG) and free fatty acid (FFA) responses in females with long-term diabetes
(GH = ng./ml., BG = mg./100 ml., FFA = μEq./L.)
Arginine infused from 0 to 30 and 80 to 110 minutes

		Time						
Patient		80	100	120	140	160	180	120
M.D.	GH	64.0	64.0	32.0	21.0	13.2	13.6	18.0
	BG	183	190	208	213	214	212	208
	FFA	540	520	630	670	730	780	920
L.J.	GH	3.6	5.0	12.4	28.0	7.2	3.0	3.8
	BG	282	285	303	315	303	312	303
	FFA	1050	1020	1040	810	1040	—	990
M.S.	GH	10.6	18.0	21.0	10.6	7.2	4.2	5.0
	BG	246	262	278	282	286	282	268
	FFA	860	670	750	920	1310	1010	710
K.M.	GH	52.0	64.0	58.0	38.0	11.6	10.6	11.6
	BG	206	224	248	274	286	282	274
	FFA	580	530	820	700	1000	920	730
J.M.	GH	21.0	32.0	21.0	9.2	7.2	3.6	1.8
	BG	270	270	284	286	284	276	268
	FFA	1090	1160	1390	1480	1700	1610	1710
M.E.	GH	25.0	16.0	11.6	7.6	9.2	10.6	—
	BG	294	296	292	300	292	286	—
	FFA	360	380	420	580	470	490	—
L.C.	GH	13.6	13.6	9.2	6.0	3.2	1.6	1.2
	BG	—	284	300	318	324	318	306
	FFA	780	950	920	1050	1050	1110	970
C.S.	GH	12.4	4.8	4.4	4.2	4.2	2.8	3.0
	BG	324	322	322	332	326	322	320
	FFA	1360	1270	1220	1110	1410	1280	1190
Mean	GH	25.2± 7.5	27.1± 8.5	21.2± 6.0	15.5± 4.3	7.8± 1.2	6.2± 1.6	6.3± 2.3
(± S.E.M.)	BG	257 ± 18	265 ± 14	279 ± 12	290 ± 13	289 ± 12	286 ± 12	278 ± 14
	FFA	827 ±117	812 ±116	898 ±111	915 ±103	1088 ±136	1028 ±135	1031 ±128

arginine infusion. This seems to indicate a subtle feedback mechanism, the purpose of which could be to prevent oversecretion when baseline secretion is high. The delayed inverse effect of baseline GH level was still present in the short-term diabetic group, but could not be detected in diabetics with long-term disease.

The positive correlation of fasting BG levels with the height of the peak GH response after the first infusion is a further indication of a regulatory system in normals. The negative correlation of fasting BG with the second GH response is in line with the effect that fasting BG exerts on the baseline GH level which in turn has a delayed effect during the second stimulus, as mentioned above.

Fasting BG levels in the subjects with short-term diabetes did not correlate with GH response after either stimulus. With long-term diabetes, the effect of fasting BG on the second response was the opposite of that in normals, i.e. there was a negative correlation between fasting BG and both the first and second GH responses. This means that in the long-term diabetic group, patients with higher fasting BG values had proportionately less GH response to arginine stimulation regardless of their baseline GH levels. This was not observed in short-term diabetics as a group. If all diabetics were divided in two groups with low and high fasting BG, the blunting effect of high fasting BG on GH secretion again became obvious.

The amount of arginine is an important factor in determining the magnitude of GH response in normals. The significant correlation between infused arginine per kilogram body weight and GH response observed in normals was not present in either diabetic group. This suggests an impaired dose response sensitivity of the pituitary in diabetics. It is considered well established that rising BG suppresses the secretion of GH in healthy persons.[27,28] But Merimee et al. could not abolish GH response to arginine by acutely elevating

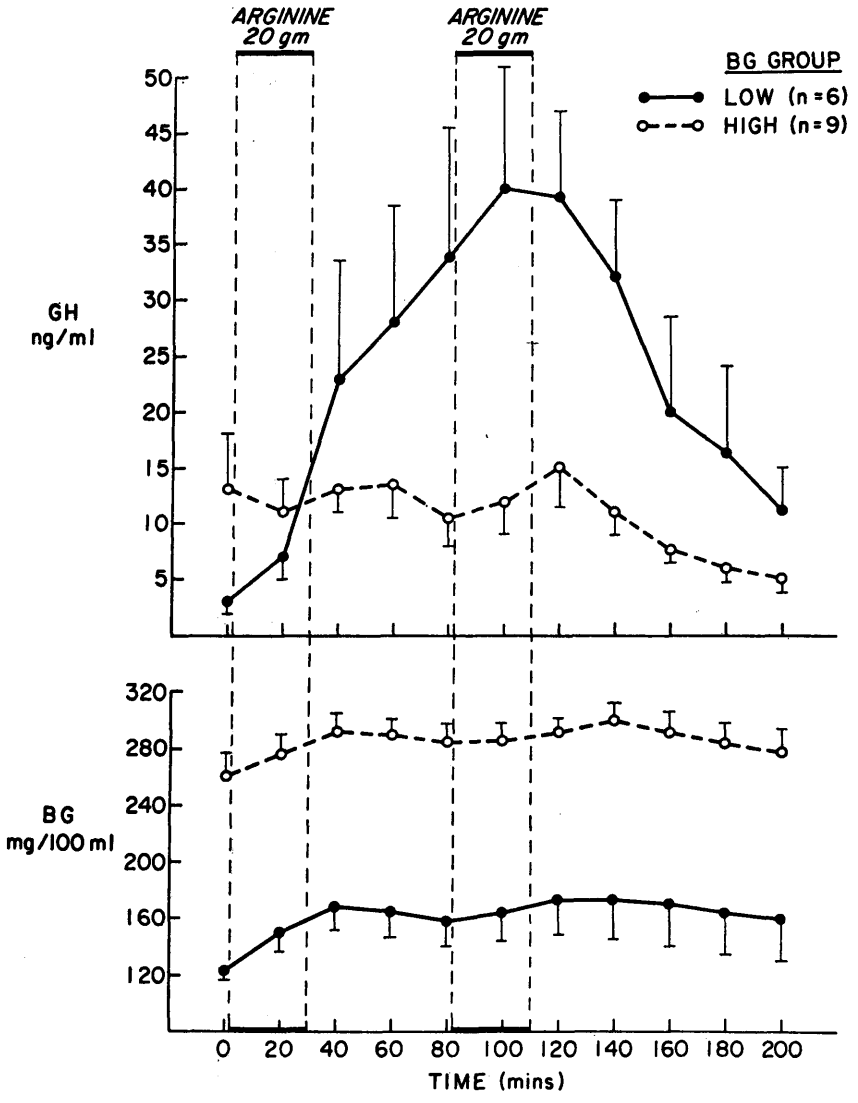

FIGURE 2

Comparisons of mean (± S.E.M.) serum growth hormone (GH) and blood glucose (BG) levels between diabetics with low and high blood glucose. The mean GH levels are significantly different at 80 ($p < 0.05$), 100 ($p < 0.02$), 120 and 140 minutes ($p < 0.01$). All BG levels were significantly different ($p < 0.05$).

BG levels in normals.[13] Burday et al. showed that hyperglycemia abolished the GH response to arginine in nine of ten normals.[23] In our study, diabetic patients with marked fasting hyperglycemia had a significantly lower GH response than patients with normal or moderately elevated fasting BG concentrations. The increment of BG rise during the test had no effect on GH response in either normals or diabetics. It is concluded that the BG rise during the test does not affect GH response, whereas the degree of hyperglycemia in the subject before the infusion is an important determinant of the GH response.

Amelioration of diabetic retinopathy after induced hypopituitarism has raised the question as to whether this complication develops in diabetics with excessive GH secretion. Powell et al.[6] examined the GH levels of patients with retinopathy and found resting GH levels within normal limits and, in addition, noted that these patients did not show expected GH elevations one hour after intravenous injection of insulin. A more recent study has shown that fasting levels of GH are significantly greater in diabetic females with retinopathy when compared with female diabetics without retinopathy and with normal subjects.[26] In this study comparison of the GH responses of five diabetic patients with retinopathy and five others without this complication showed that the mean fasting GH levels were similar and, after the arginine stimulations, the GH levels were lower in the group with retinopathy. The higher mean BG levels in the retinopathy group may account

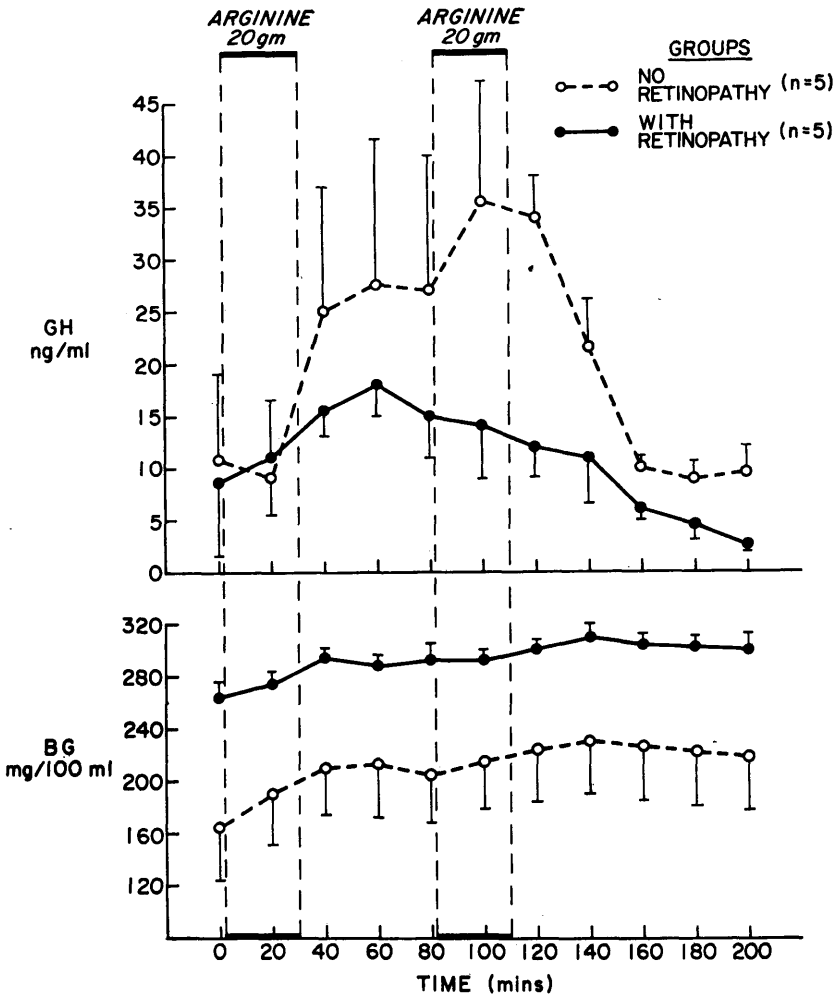

FIGURE 3

Comparisons of mean (± S.E.M.) serum growth hormone (GH) and blood glucose (BG) levels in patients with and without retinopathy. The mean GH levels are significantly different at 120 ($p < 0.02$), 160 and 200 minutes ($p < 0.05$). Differences in the BG levels did not achieve significance.

for the lower GH responses in these patients, as pointed out earlier. Although the number of patients studied is relatively small, the over-all trend in the retinopathy group is a reduced GH response to arginine.

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REFERENCES

[1] Ikkos, D., and Luft, R.: "Idiohypophyseal" diabetes mellitus in two hypophysectomised women. Lancet *2*:897-99, 1960.

[2] Young, F. G.: Growth hormone and diabetes mellitus. Nord. Med. *78*:950, 1967.

[3] Roth, J., Glick, S. M., Cuatrecasas, P., and Hollander, C. S.: Acromegaly and other disorders of growth hormone secretion. Ann. Intern. Med. *66*:760-88, 1967.

[4] Oakley, W. G.: Aetiology of diabetes mellitus and its complications. *In* Ciba Foundation Colloquia on Endocrinology, vol. 15, Cameron, M. P., and O'Connor, M., editors. London, Churchill, 1964, pp. 356-57.

[5] Field, R. A.: Approaches to treatment of retinopathy by modifying pituitary function. *In* On the Nature and Treatment of Diabetes, Leibel, B. S., and Wrenshall, G. A., editors. Amsterdam, Excerpta Medica Foundation, 1965, p. 487.

[6] Powell, E. D. U., Frantz, A. G., Rabkin, M. T., and

Field, R. A.: Growth hormone in relation to diabetic retinopathy. N. Engl. J. Med. *275*:922-25, 1966.

[7] Penny, R., Blizzard, R. M., and Davis, W. T.: Sequential study of arginine monochloride and normal saline as stimuli to growth hormone release. Metabolism *19*:165-69, 1970.

[8] Best, J., Catt, K. J., and Burger, H. G.: Non-specificity of arginine infusion as a test for growth hormone secretion. Lancet *2*:124-26, 1968.

[9] Baker, H. W. G., Best, J. B., and Burger, H. G.: Arginine-infusion test for growth hormone secretion. Lancet *3*: 1193, 1970.

[10] Boden, G., and Soeldner, J. S.: A sensitive double antibody radioimmunoassay for human growth hormone (HGH): Levels of serum HGH following rapid tolbutamide infusion. Diabetologia *3*:413-21, 1967.

[11] Hoffman, W. S.: Rapid photoelectric method for determination of glucose in blood and urine. J. Biol. Chem. *120*: 51-55, 1937.

[12] Dole, V. P., and Meinertz, H.: Microdetermination of long chain fatty acids in plasma and tissues. J. Biol. Chem. *235*:2595-99, 1960.

[13] Merimee, T. J., Rabinowitz, D., and Fineberg, S. E.: Arginine-initiated release of human growth hormone. N. Engl. J. Med. *280*:1434-38, 1969.

[14] Boden, G., Soeldner, J. S., Gleason, R. E., and Marble, A.: Elevated serum growth hormone and decreased serum insulin in prediabetic males after intravenous tolbutamide and glucose. J. Clin. Invest. *47*:729-38, 1968.

[15] Sonksen, P. H., Boden, G., Gleason, R. E., and Soeldner, J. S.: "Inappropriate" growth hormone elevation in prediabetic males. J. Clin. Invest. *47*:92a, 1968.

[16] Knopf, R F.. Fajans, S. S, Floyd, J. C., Jr., Pek, S., and Conn, J. W.: Growth hormone responses of healthy subjects, healthy relatives of diabetic patients, and mildly diabetic subjects to administration of amino acids or protein meals. Diabetes *18*(Suppl. 1):334, 1969.

[17] Sabeh, G., Corredor, D. G., Mendelsohn, L. V., Morgan, C. R., Sieracki, J. C., Sunder, J. H., Wingert, J. P., and Danowski, T. S.: Growth hormone and insulin levels in newly discovered glucose intolerance. Metabolism *18*:741-47, 1969.

[18] Parker, M. L., Hammond, J. M., and Daughaday, W. H.: The arginine provocative test: An aid in the diagnosis of hyposomatotropism. J. Clin. Endocrinol. Metab. *27*:1129-32, 1967.

[19] Drash, A., Field, J. B., Garces, L. Y., Kenny, F. M., Mintz, D., and Vasquez, A. M.: Endogenous insulin and growth hormone response in children with newly diagnosed diabetes mellitus. Pediatr. Res. *2*:94-102, 1968.

[20] Baker, L., Root, A. W., Haque, N., and Kaye, R.: Metabolic homeostasis in juvenile diabetes mellitus. I. Role of growth hormone. Metabolism *18*:110-14, 1969.

[21] Johansen, K., and Hansen, A. P.: High 24-hour level of serum growth hormone in juvenile diabetics. Br. Med. J. *2*: 356-57, 1969.

[22] Hansen, A. P..: Abnormal serum growth hormone response to exercise in juvenile diabetics. J. Clin. Invest. *49*: 1467-78, 1970.

[23] Burday, S. Z., Fine, P. H., and Schalch, D. S.: Growth hormone secretion in response to arginine infusion in normal and diabetic subjects: Relationship to blood glucose levels. J. Lab. Clin. Med. *71*:897-911, 1968.

[24] Merimee, T. J., Burgess, J. A., and Rabinowitz, D.: Arginine infusion in maturity-onset diabetes mellitus. Lancet *1*: 1300-01, 1966.

[25] Lundbaek, K., Christensen, N. J., Jensen, V. A., Johansen, K., Olsen, T. S., Hansen, A. P., Orskov, H., and Osterby, R.: Diabetes, diabetic angiopathy, and growth hormone. Lancet *2*:131-33, 1970.

[26] Knopf, R. F., Fajans, S. S., Floyd, J. C., Jr., Pek, S., and Conn, J. W.: Elevated "casual" fasting plasma levels of growth hormone (GH) in patients with diabetic retinopathy (DR). Diabetes *21*(Suppl. 1):322, 1972.

[27] Beck, P., Schalch, D. S., Parker, M. L., Kipnis, D. M., and Daughaday, W. H.: Correlative studies of growth hormone and plasma insulin concentrations with metabolic abnormalities in acromegaly. J. Lab. Clin. Med. *66*:366, 1966.

[28] Boden, G., Soeldner, J. S., Steinke, J., and Thorn, G. W.: Serum human growth hormone (HGH) response to intravenous glucose: Diagnosis of acromegaly in females and males. Metabolism *17*:1-9, 1968.