Plasma Growth Hormone Concentration in Corticosteroid-Treated Children

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ABSTRACT Endogenous plasma growth hormone concentrations were measured in 23 children who were receiving daily corticosteroid therapy and in 10 control asthmatic children who had not received steroids for at least 8 months. The growth hormone concentrations were similar in the two groups of patients both during the fasting state and after insulin-induced hypoglycemia. 12 children, who were studied while receiving a large dose of prednisone and again 2 wk after steroid withdrawal, also showed no change in growth hormone concentration in relation to corticosteroid therapy. These findings suggest that deficiency of growth hormone is not the major mechanism responsible for the dwarfism of corticosteroid-treated children.

INTRODUCTION

The mechanisms responsible for growth retardation in children given prolonged corticosteroid therapy have not been defined. Previous studies in this laboratory have demonstrated that corticosteroid-treated children have impaired metabolic response to acute administration of human growth hormone (HGH) and show no improvement in their retarded rate of growth during prolonged HGH administration (1). These observations have suggested that the mechanism responsible for the growth retardation might be due to corticosteroid-induced antagonism of the effects of HGH at the tissue level. On the other hand, Frantz and Rabkin and Hartog, Gaafar, and Fraser have reported that corticosteroid-treated adults show inhibition of pituitary release of GH after hypoglycemia (2, 3) and have postulated that dwarfism in steroid-treated children might be due in part to relative deficiency of endogenous GH (2). The present study was undertaken to determine plasma growth hormone levels in relation to corticosteroid treatment in a relatively homogeneous population of children who suffer from asthma. Plasma growth hormone levels in steroid-treated children during the fasting state and after induced hypoglycemia were compared with those observed in nonsteroid-treated asthmatic control children. Many of the children were studied both during corticosteroid administration and after total withdrawal of steroids.

METHODS

Patient population. Studies were performed on 33 children, aged 8–15, who were resident patients of the Children's Asthma Research Institute and Hospital (CARIH). 10 control asthmatic children had received no corticosteroid for at least 8 months. 23 children had received daily corticosteroid for prolonged periods with therapy at the dose indicated for at least 1 month. 11 of the children required maintenance with stable doses of steroid (prednisone 2.5–30 mg/day). 12 children were able to tolerate total discontinuation of steroid and were studied while receiving a large dose of prednisone (10–15 mg/day) and again 2 wk after steroid withdrawal. Some of the patients in the last group were studied on several occasions as steroid dose was being reduced (by 2.5 mg every 10 days) and at intervals after discontinuation of steroid. The sex distribution of the population at CARIH is reflected in our study, with the steroid-treated group containing a larger percentage of males and the nonsteroid group containing a higher percentage of females.

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Study protocol. All studies were performed on fasting subjects who had been at bed rest for at least 2 hr. Most of the children receiving corticosteroid had their usual morning dose of prednisone withheld until completion of the test. After initial venipuncture, an intravenous infusion of normal saline was begun and continued throughout the study. All subsequent blood samples were obtained through the infusion needle after the first few drops of blood were discarded to minimize dilution error. Two control samples were obtained for glucose and GH determinations. Crystalline insulin was then administered intravenously at a dose of 0.1 U/kg of body weight. Repeat samples for glucose determination were obtained 20, 30, 45, 60, 90, and 120 min after insulin administration. Repeat samples for GH determination were obtained 30, 60, 90, and 120 min after insulin administration. Some studies were terminated before 120 min because of persisting hypoglycemia.

A physician remained in the room continously throughout each insulin tolerance test. Glucagon and 50% glucose were immediately available to counteract hypoglycemia if this seemed indicated. At the termination of the study, children were given sweetened juice and were fed. Close observation was continued for several hours.

Methods of analysis. Samples for blood glucose were collected in tubes containing sodium fluoride and were analyzed by either the glucostat method (4) or by the AutoAnalyzer (5). The values reported are the fasting sugars, the minimum blood sugars (obtained at either 20 or 30 min after insulin administration), and the 60-min values.

Samples for GH were collected in heparinized tubes, kept in an ice bath until separated in a refrigerated centrifuge, and then stored in a freezer. GH concentration was determined by the radioimmunoassay method of Glick, Roth, Yalow, and Berson (6). Most of the plasma samples were assayed on several occasions, and the value shown is the mean of all determinations. The GH values reported are those observed during the fasting state and 60 min after insulin administration. The 60 min sample was selected as the indicator of response to hypoglycemia, since this value was determined in all patients studied and in most subjects was the highest value observed. Glucose and GH results obtained in the different groups of children were analyzed by Student's t test (7).

Immunoassay method. ^{131}I-labeled HGH (specific activity 300–500 μc/μg) was prepared by the method of Greenwood, Hunter, and Glover (8) using 5 μg of HGH (Wilhelmi HS 612B), 2–3 mc of ^{131}I (Iso-Serve, Cambridge Nuclear Corp., Cambridge, Mass.), and 44 μg of chloramine-T for 1 min, followed by 120 μg of sodium metabisulfite. The labeled fraction containing the least amount of damage after purification (starch gel electrophoresis (9) or filtration through a Sephadex G-100 column (10)) was identified by paper chromatoelectrophoresis (11) and was used in the assay after dilution to 60,000 cpm. Antiserum prepared in rabbits using Elrick HGH (E-20) (12) was adsorbed by precipitation with lyophilized, pooled human serum to remove possible antibody to human serum components and was used in the assay at a final concentration of 1 : 130,000.

Standard curves were prepared for each immunoassay run using either Wilhelmi GH (HS 612B) or the British Medical Research Council (MRC) "GH assay standard A" which gave identical slopes.[1] Unknown samples were calculated on the basis of the MRC standard. Each plasma sample was assayed in duplicate at three different dilutions, with one tube in each set of three serving as a control tube. 0.1 ml of plasma or diluted plasma was used in the incubate. All solutions were prepared with Veronal buffer 0.1 mole/liter, pH 8.6, containing 1% normal rabbit serum and 2.5 mg/ml of human serum albumin (The Cutter Laboratories, Berkeley, Calif.). Tubes containing either standards or plasma samples were incubated with labeled GH ^{131}I and antiserum in a final volume of 1 ml for 4–8 days at 5°C. A 0.2 ml aliquot of the incubate (and 0.02 ml of pooled human serum for carrier protein in incubates containing standards or diluted plasma specimens) were then applied to 1¼-inch wide strips of Whatman 3MC chromatographic paper and subjected to chromatoelectrophoresis at 30 v/strip for 45 min at room temperature, which separated the radioactive label into a free peak (F) and an antibody-bound peak (B). Paper strips were oven-dried and then scanned for radioactivity at the rate of 2 cm/min in an automatic 4 pi radiochromatograph scanner equipped with an integrator recorder. The integrator excursions were used to determine the areas under radioactivity peaks.

Standard curves were plotted either on linear graph paper as B/F ratio vs. concentration of HGH in millimicrograms per milliliter, according to the method of Berson-Yalow (11), which gave the curve shown in Fig. 1, or were plotted on semilogarithmic paper as per cent bound vs. log GH concentration which gave the linear relationship shown in Fig. 2. The latter plot was found to be more convenient for estimating the GH concentration of unknown plasma samples.

Precision of the immunoassay. Since the GH values reported here were obtained by duplicate determinations at several dilutions and in most instances were analyzed on several occasions, the results obtained with these plasma samples were used to estimate the precision of the immunoassay in our hands. The mean difference in per cent of radioactivity in the bound fraction in 400 duplicate determinations (at various dilutions) was ± 3%. In terms of millimicrograms per milliliter of GH, comparison of 182 duplicate determinations which yielded measurable GH values showed a mean difference of ± 22%. The precision at various ranges of GH concentration shown in Table I is similar to that reported for GH immunoassay by other investigators (13–15). Comparison of the results obtained when 148 specimens were analyzed at two different dilutions indicated a slight but significantly greater value at the higher dilution. Comparison of the results observed when 115 specimens were analyzed on different occasions yielded no significant differences.

[1] We are indebted to Dr. A. E. Wilhelmi (National Pituitary Agency) and Dr. D. Bangham (British Medical Research Council) for providing the purified GH preparations used for assay standards.

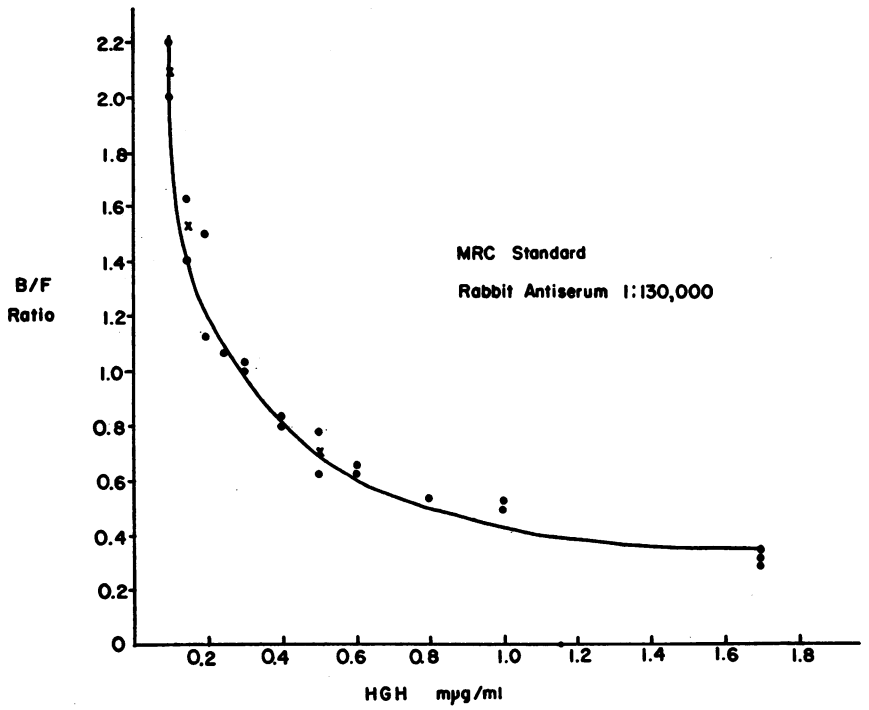

FIGURE 1 Growth hormone immunoassay standard curve (2/9/66). The results are plotted as antibody-bound peak/free peak ratio (B/F ratio) vs. GH concentration (mμg/ml).

RESULTS

We found that induction of hypoglycemia for the purpose of measuring plasma GH concentrations was a safe procedure in the asthmatic children. A single, potentially serious reaction was encountered when one child developed Cheyne-Stokes respirations 30 min after insulin administration with a blood glucose concentration of 25 mg/100 ml. He was immediately given intravenous glucose and recovered promptly; subsequent observation revealed no sequellae. Not one acute episode of asthma occurred during the conduct of these studies, and if anything, children had less severe asthma during the hours after hypoglycemia.

TABLE I

Precision of GH Immunoassay

Range GH concentration	No. of samples	Mean GH concentration	Standard deviation*
mμg/ml		*mμg/ml*	
1–9	54	6.3	±1.5 (24%)
10–19	65	15.3	±4.0 (26%)
20–29	32	24.1	±5.1 (21%)
30+	31	48.8	±7.0 (14%)

* SD = $\sqrt{D^2/2N}$ (16).

Table II lists the pertinent clinical features of the patients and the results of the glucose and GH determinations. It can be seen that most of the children, steroid-treated as well as control subjects, had measurable fasting levels of GH and that in most the concentration of GH rose after insulin administration.

In both steroid-treated and control groups very high fasting levels of GH were occasionally encountered despite the long rest period before collection of initial samples. In some instances, the high values occurred in children who were noted at the time of the test to be particularly apprehensive or upset. Most of the high values were encountered in females. As noted previously and indicated in Table II, the control group contains a much higher ratio of females than the steroid groups, which may account for the higher mean fasting value.

Fig. 3 graphically illustrates the GH values observed in children during steroid treatment,

TABLE II

Glucose and GH Values during Insulin Tolerance Tests

Patient	Age	Sex	Steroid* dose	Glucose			GH	
				0	Minimum‡	60 min§	Fasting	60 min
			mg	*mg/100 ml*			*mμg/ml*	
Nonsteroid-treated control children								
A. H.	13	M	0	95	22	53	2.5	23
G. L.	13	M	0	88	20	42	6	20
T. L.	8	M	0	94	20	58	1.5	7
D. P.	13.5	M	0	87	19	47	10	27
K. A.	11	F	0	82	26	45	4	54
B. B.	12	F	0	88	37	59	32.5	21
R. C.	12	F	0	97	—	81	4.5	18
K. H.	11	F	0	87	28	78	15	20
M. R.	10	F	0	90	30	67	22	29
R. S.	13	F	0	87	24	38	23	31
Children during steroid treatment and 2 wk after steroid withdrawal								
M. C.	15	M	15	100	27	72	3	81
			0	93	32	56	1	78
S. E.	9	M	10	88	28	64	4	10
			0	89	35	46	10	20
M. G.	9.5	M	10	86	37	72	2.5	30
			0	57	28	25	4.1	22
D. G.	10.5	M	10	87	27	60	9	15
			0	91	32	53	5	9
W. I.	7.5	M	7.5	79	40	62	12	13
			0	74	22	38	20	18
S. K.	14	M	10	95	20	67	7.5	30
			0	88	27	61	4.1	50
B. L.	12	M	10	82	52	80	5.7	58
			0	87	16	62	5	21
J. M.	11	M	15	87	43	85	4	14
			0	85	32	57	19	22
G. P.	12	M	10	89	32	62	6	18.5
			0	69	32	38	3	22
L. C.	12	F	10	83	32	77	24	26
			0	85	27	58	1.5	22
S. F.	9	F	10	74	30	52	7	12
			0	81	32	36	6.5	21
B. S.	12	F	10	97	46	72	3.5	29
			0	84	47	66	3	73
Children on stable dose steroid								
F. G.	15	M	2.5	73	28	65	5	46
C. H.	9	M	5	90	21	62	6	4
B. K.	11	M	10	89	32	58	1	20
L. L.	13	M	30	98	36	77	8.5	12
P. L.	13	M	15	86	26	62	14	12
D. N.	11	M	10	78			10.5	11
R. R.	15	M	10	88	31	61	1	25
J. S.	13	M	20	83	32	71	11	22
C. B.	13	F	5	100	29	56	9	5.8
D. B.	15	F	10	78	42	55	14	15
L. G.	12	F	5	81	35	54	24	19

* Daily dose of prednisone.

‡ Minimum refers to minimum plasma concentration after insulin.

§ 60 min refers to glucose and GH concentration 60 min after insulin.

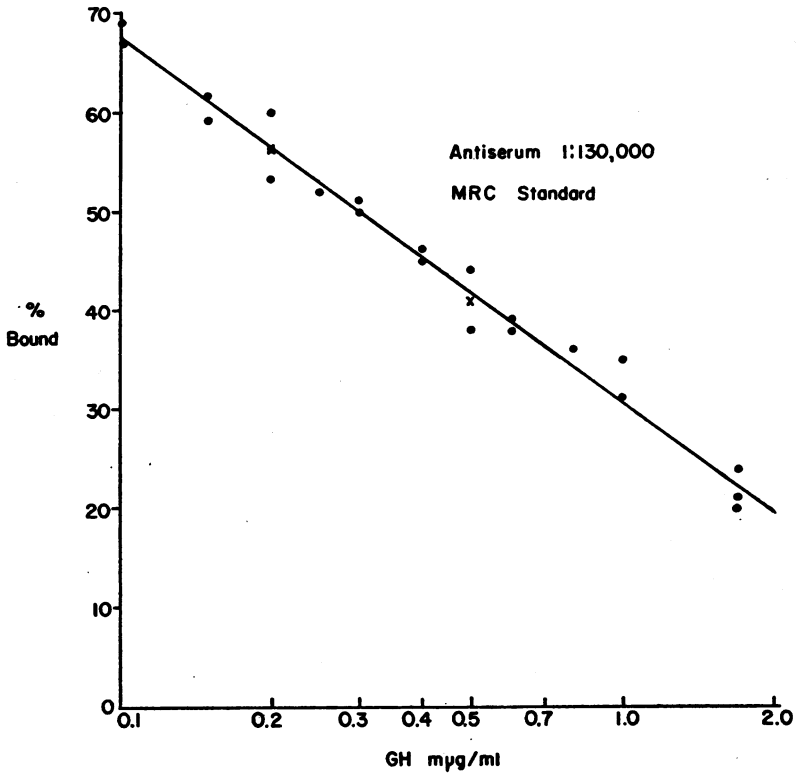

FIGURE 2 Growth hormone immunoassay standard curve (2/9/66). The same data shown in Fig. 1 are plotted as per cent of bound radioactive label vs. log GH concentration in mμg/ml.

shortly after steroid withdrawal, and in control subjects. Although there was considerable variability, the mean concentrations and range of values were quite similar in the three groups both during the fasting state and after induced hypoglycemia. (Mean fasting GH concentration: control = 12.1 mμg/ml; steroid-treated = 8.4 mμg/ml; steroid-withdrawn = 6.9 mμg/ml. Mean postinsulin GH concentration: control = 25 mμg/ml; steroid-treated = 23 mμg/ml; steroid-withdrawn = 31.4 mμg/ml.). In each of the groups the rise in plasma GH concentration after insulin administration was significant ($P = 0.01$–0.05). The postinsulin values in the steroid-treated children showed more variability than that observed in either of the control groups. With the exception of the two lowest postinsulin values (observed in patients who were in the stable dose steroid group and who received a small dose of prednisone on the morning of the study), the distribution of values was similar in children who subsequently tolerated withdrawal of steroid and those who did not.

Fig. 4 compares the GH concentrations in individual children who were studied while receiving a large dose of prednisone and again 2 wk after corticosteroid withdrawal. The similarity of values is further shown by the data in Table III which lists the GH concentrations observed in several children who were studied serially while steroid dosage was being reduced and at intervals after total withdrawal of corticosteroid. It can be seen that the GH values for each individual child were fairly reproducible and that there was no consistent trend in either fasting or postinsulin values in relation to discontinuation of steroids.

Table IV summarizes the results of statistical comparison of the glucose and GH values observed in the different groups of children. There were no significant differences in GH values in children recently withdrawn from steroid compared with control children who had not received steroid for many months. The standard dose of insulin (0.1 U/kg) produced significantly lower minimum and 60-min blood sugars in contol sub-

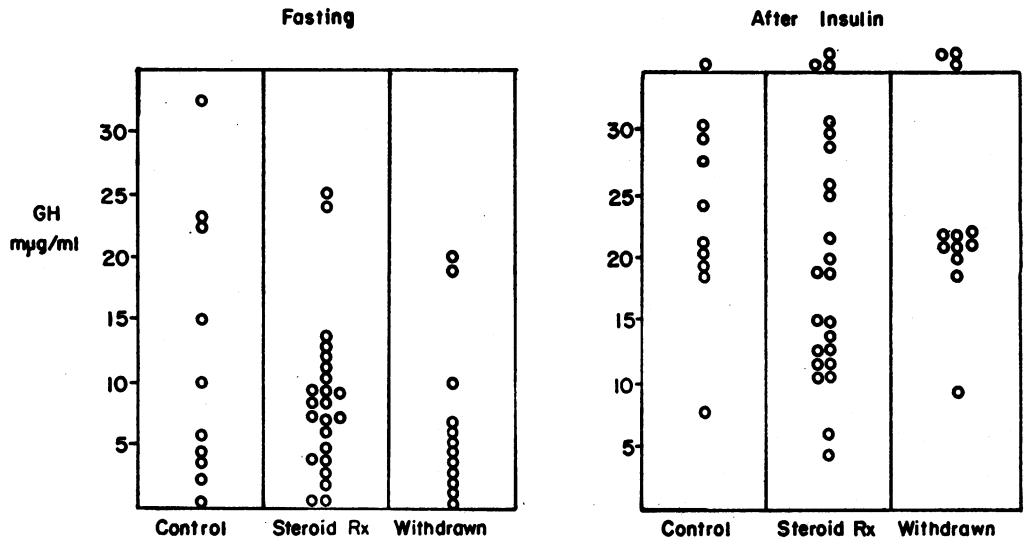

FIGURE 3 Plasma GH concentrations in control and steroid-treated children. Each open circle represents a single patient.

jects than those observed in children who were receiving steroid therapy ($P = <0.05$). Despite the differences in degree and duration of hypoglycemia, there was no significant difference in mean GH values during either the fasting state or after insulin administration in steroid-treated and control children (fasting GH $P = 0.2$; post-insulin GH $P = 0.65$). Similarly, comparison of the results observed when the same subjects were studied during steroid therapy and shortly after steroid withdrawal again revealed no significant differences in GH values ($P = 0.60$–0.85), despite the fact that during the poststeroid withdrawal period the duration of hypoglycemia was significantly greater ($P = <0.001$ for blood glucose at 60 min).

The GH value at 60 min after insulin administration was used as the index of response to hypoglycemia in comparing the various groups of subjects, since this value was measured in all studies and most subjects exhibited maximum GH concentration at this time. Data at later points after insulin administration are less complete, since a number of studies were terminated

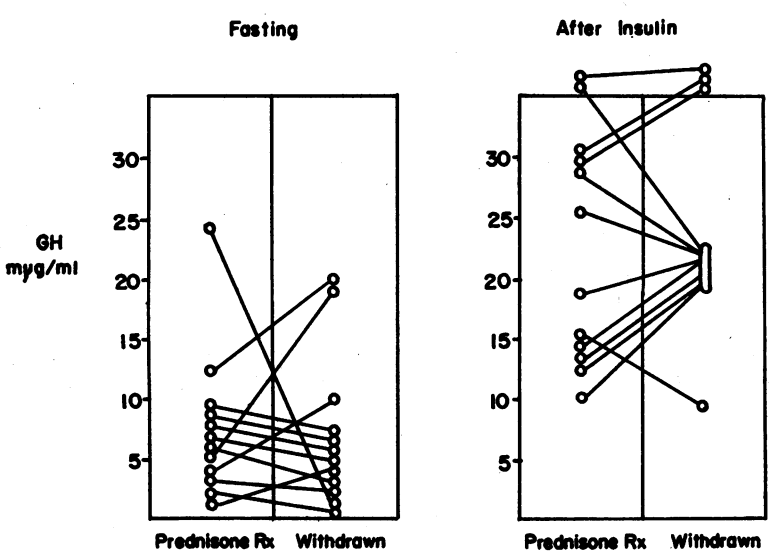

FIGURE 4 Plasma GH levels in individual children during steroid treatment and 2 wk after steroid withdrawal. The connecting bars identify the GH values observed in each child during treatment and after steroid withdrawal.

TABLE III

GH Levels during Prednisone Therapy and after Withdrawal

	Daily prednisone dosage				Duration of withdrawal		
	15 mg	10 mg	5 mg	2.5 mg	2 wk	6 wk	10 wk
Patient							
L. C.		24*		4	1.5	3	11
		26‡		15	22	15	21
M. G.		2.5		3.1	4.1		
		30		26	22		
D. G.		9		5.5	5		2
		15		15	9		14
J. M.	4	4		2.6	19	9.5	
	14	23		42	22	21	
M. C.	3				1	3	4.5
	81				78	127	113

* Upper number of each pair = fasting GH value.
‡ Lower number of each pair = value after insulin.

before 2 hr because of persistent symptoms of hypoglycemia. However, available specimens revealed that mean GH concentrations at 90 min were similar to those observed at 60 min in each group. While most subjects showed a beginning decrease in GH concentration, there were a few subjects in each group who showed a further rise in GH level at 90 min. By 120 min the GH con-

TABLE IV

Statistical Comparison of Plasma Glucose and Growth Hormone Concentrations in Steroid-Treated and Control Patients

	Mean glucose			Mean GH		
	mg/100 ml			*mμg/ml*		
Recent steroid withdrawal vs. control						
	Steroid withdrawal (n = 12)	Control (n = 10)		Steroid withdrawal (n = 12)	Control (n = 10)	
Fasting	81.9 ± 10.4	89.5 ± 4.5	P = 0.05	6.9 ± 6.3	12.1 ± 10.6	P = 0.15
Minimum*	30.2 ± 7.5	25.1 ± 5.8	P = 0.11			
60 min‡	49.7 ± 12.8	56.8 ± 14.8	P = 0.25	31.4 ± 22.7	25 ± 12.2	P = 0.45
Steroid-treated vs. control						
	Steroid-treated (n = 23)	Control (n = 10)		Steroid-treated (n = 23)	Control (n = 10)	
Fasting	86.6 ± 7.6	89.5 ± 4.5	P = 0.3	8.4 ± 6	12.1 ± 10.6	P = 0.2
Minimum	33 ± 7.7	25.1 ± 5.8	P = 0.02			
60 min	65.7 ± 8.8	56.8 ± 14.8	P = 0.04	23 ± 17.4	25 ± 12.2	P = 0.75
Same children during steroid treatment and after steroid withdrawal						
	On steroid (n = 12)	Off steroid (n = 12)		On steroid (n = 12)	Off steroid (n = 12)	
Fasting	87.2 ± 7.5	81.9 ± 10.4	P = 0.17	7.4 ± 5.9	6.9 ± 6.3	P = 0.85
Minimum	34.5 ± 9.3	30.2 ± 7.5	P = 0.23			
60 min	68.8 ± 9.3	49.7 ± 12.8	P = 0.001	28 ± 21.3	31.4 ± 22.7	P = 0.6

* Minimum refers to minimum plasma glucose concn after insulin.
‡ 60 min refers to glucose and GH concentrations 60 min after insulin.

centrations were approaching preinsulin levels and again were higher in control subjects than in corticosteroid-treated or withdrawn groups. At 90 and 120 min blood glucose was similar in control and steroid-treated children but continued to be lower in subjects recently withdrawn from steroid.

DISCUSSION

Corticosteroid inhibition of pituitary GH release has been reported in adults and postulated as the mechanism responsible for growth inhibition in steroid-treated children (2). In the present study, plasma GH concentrations were measured in 33 asthmatic children including 23 who had received prolonged corticosteroid therapy. The data indicate that corticosteroid-treated children secrete growth hormone and maintain plasma GH concentrations which are similar to those of nonsteroid-treated control subjects, both during the fasting state and after induced hypoglycemia. GH values in individual children studied during therapy and after steroid withdrawal were found to be fairly reproducible and showed no differences in relation to corticosteroid administration. The plasma growth hormone concentrations reported here are similar to the values which have been observed in other children (17–19).

The protocol used in the present study differed from those used by other investigators (2, 3) for studying corticosteroid-treated subjects. In contrast to previous studies (2, 3) in which plasma GH response to induced hypoglycemia was evaluated within 1–2 hr after corticosteroid administration, most of the present studies were performed approximately 12–16 hr after the last dose of steroid (prednisone). Our data therefore provide no information about the acute effects of corticosteroid on pituitary function. However, the data clearly indicate that prolonged suppression of pituitary GH release does not occur in the steroid-treated children. Since maintenance corticosteroid therapy is usually administered within an 8–12 hr daytime period and allows a 12–16 hr interval between the last evening dose and the first morning dose, our results suggest that corticosteroid-treated children are capable of maintaining normal plasma GH levels during a considerable portion of the day.

The demonstration of normal plasma GH levels in children receiving therapy in doses known to be associated with growth retardation (20, 21) argues against deficiency of GH as the primary mechanism responsible for dwarfism. Similar studies have shown that children with other nonpituitary forms of dwarfism also have normal levels of GH during fasting and after hypoglycemia (17–19). However it is recognized that pituitary release of GH in response to an artificial, severe hypoglycemic stimulus may not be comparable to GH release under more normal conditions. It still remains to be demonstrated whether the mean daily plasma concentration to which tissues are exposed is similar in corticosteroid and other dwarfed children as compared with normal controls.

It was of interest that the mean GH values were similar in steroid-treated and control children despite significant differences in degree and duration of hypoglycemia. It has been shown in adults that small doses of insulin may not produce sufficient hypoglycemia to cause maximal release of pituitary GH (2, 22). On the other hand, the data presented by Greenwood, Landon, and Stamp suggest that there is a maximal stimulus, above which greater degree or duration of hypoglycemia does not result in further rise in plasma GH concentration (22). The results of the present study suggest that the degree of hypoglycemia achieved in the steroid-treated subjects is sufficient to cause maximal release of GH in most children, and that more severe hypoglycemia represents a supramaximal stimulus. It is possible, however, that the lower postinsulin GH values observed in some steroid-treated children are related to the lesser degree of hypoglycemia in this group.

The current study further emphasizes the difference in magnitude of GH levels in children as compared with adults. Adults have low fasting levels of GH (< 5 mμg/ml) and show a brisk response to hypoglycemia with postinsulin GH values of > 40 mμg/ml (23). In contrast the children in this study, as well as those reported by other investigators (17–19), have higher fasting levels of GH (6–8 mμg/ml) and show a smaller magnitude of rise in GH level (15–30 mμg/ml) after hypoglycemia. Data reported by Cornblath, Parker, Reisner, Forbes, and Daughaday have shown that young infants have even higher fasting GH levels (15–60 mμg/ml) (24). These studies indicate that plasma GH levels can be expected to vary with age

and suggest that some of the mechanisms that control pituitary GH release may be different in children and in adults.

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