Responsiveness of Growth Hormone-Deficient Children to Human Growth Hormone

EFFECT OF REPLACEMENT THERAPY FOR ONE YEAR

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ABSTRACT Previous studies have shown that growth hormone (GH)-deficient children are more responsive to exogenous human growth hormone (HGH) than non-GH-deficient children. In six GH-deficient children, velocity of linear growth was less than 2.5 cm/yr. By the metabolic balance study technique, anabolic responses (increments in elemental balances) were measured to a 7 day course of 0.0532 U HGH/kg body weight $(BW)^{3/4}$ per day (dose B) and to 0.168 U/kg $BW^{3/4}$ per day (dose C). They were then treated for 1 yr with HGH at a dose intermediate between B and C. Velocity of linear growth accelerated to 15–25 cm/yr for the first 4–7 mo, then declined to 0–8 cm/yr. At 12 mo, responsiveness to doses B and C was measured again; the responses were only 20–60% as great as before treatment. After 3 mo without HGH treatment, responsiveness to the anabolic effects of doses B and C returned to the magnitudes observed before treatment. A low titer of plasma antibodies to HGH was detected in two of the six children at the end of the year's treatment; these titers showed little change after 3 mo without HGH. Thus the hyperresponsiveness of GH-deficient subjects to exogenous HGH, compared to non-GH-deficient individuals, declines during long-term HGH treatment and is restored by 3 mo interruption of treatment. These changes in peripheral responsiveness may be related to the decline in velocity of linear growth which occurs after 4–7 mo of continuous treatment.

When HGH was withdrawn after 12 mo, all six patients exhibited negative balances of N, P, Na, and K and loss of BW. Ratios of elemental balances showed about half the weight loss to represent protoplasm, and about half extracellular fluid. These observations indicate a role of GH in the continuing regulation of nitrogen and mineral metabolism in addition to its function as a growth-promoting hormone.

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INTRODUCTION

The six growth hormone (GH)[1]-deficient children who are the subject of this report have been under observation since 1968. Their growth rate during 1968–1970 was < 2.5 cm/yr. In 1970 their anabolic responses were measured to a 7 day course of 0.0532 U HGH/kg $BW^{3/4}$ per day (dose B) and to 0.168 U/kg $BW^{3/4}$ per day (dose C). They were then begun on a 1 yr course of HGH at a dose intermediate between B and C. During this year, linear growth rate accelerated to 15–25 cm/yr for the first 4–7 mo, then declined to 0–8 cm/yr.

To investigate the cause of this decline, we remeasured the responsiveness of the patients to doses B and C of HGH at the end of the year's treatment and again after an additional 3 mo without GH treatment. The changes that were observed in responsiveness to HGH before, during, and after the year of hormone treatment form the basis of this report.

METHODS

Clinical data of the six GH-deficient subjects are summarized in Table I. Criteria for deficiency of endogenous GH were as previously described: growth rate less than 5 cm/yr while endogenous or exogenous glucocorticoid and thyroid hormones were available in normal amount; bone age less than 75% of chronologic age; serum HGH concentrations less than 3 ng/ml throughout two insulin and two arginine

[1] *Abbreviations used in this paper:* BW, body weight; GH, growth hormone; HGH, human growth hormone.

TABLE I

Clinical Summaries of the Subjects

Case No.	Age	Sex	Ht	Wt	Rate of growth before HGH*	Bone age	Diagnosis	Duration since diagnosis	Pituitary hormone deficiencies	Hormone replacement treatment
	yr		*cm*	*kg*	*cm/yr*	*yr*		*yr*		
1	9	M	88	15	0.8	4.5	Isolated GH deficiency (idiopathic)	7	GH‡	None
2	9	M	114	24	1.2	4.5	Isolated GH deficiency (idiopathic)	3	GH	None
3	16	M	165	46	2.4	11.5	Chromophobe adenoma; X-ray therapy	3	GH, ADH, probably FSH and LH	Pitressin
4	18	M	152	37	0.8	8.5	Adenohypophyseal insufficiency (idiopathic)	9	GH, ACTH, TSH, FSH, LH	Thyroxine, cortisol
5	17	F	134	37	1.2	14	Deficiency of GH and gonadotropins (idiopathic)	4	GH, FSH, LH	None
6	19	F	140	37	1.4	13	Panhypopituitarism after excision of cranio-pharyngioma	11	GH, TSH ACTH, FSH, LH, ADH	Thyroxine, cortisol, pitressin

* Average of 2 yr preceding treatment with HGH for 1 yr.

‡ ADH, antidiuretic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; TSH, thyroid-stimulation hormo ne.

provocative tests, these tests being preceded in male patients by 2 days of treatment with diethylstilbestrol 5 mg orally twice a day.

Measurement of anabolic response to HGH

Measurement of anabolic response to HGH was done by the metabolic balance study technique as previously described (1).

(*a*) *Before long-term treatment* (*exp. 1*). After a 3 day equilibration on the metabolic diet, a 7 day control period (*a*) was begun. This was followed by 7 days of dose B (*b*); a 7 day control period (*c*); 7 days at dose C (*d*); then 7 days of control period (*e*). Response to dose B or dose C (ΔN, ΔP, ΔNa, ΔK, ΔBW) was calculated as (average daily elemental balance during treatment period minus average balance during preceding control period) divided by (kg $BW^{3/4} \times 10^{-1}$) or as (BW at end of treatment period minus that at end of preceding control period divided by 7) divided by (kg $BW^{3/4} \times 10^{-1}$). The HGH was furnished by the National Pituitary Agency. In all phases of this investigation, HGH was injected at 11 p.m., and ACTH-deficient subjects ingested their exogenous cortisol at 7 a.m., because of the evidence (2) that the anabolic effect of HGH is enhanced by these conditions.

(*b*) *At the end of 1 yr replacement treatment* (*exp. 2*). The patient was then treated at home with 2 U HGH (Calbiochem, San Diego, Calif.) 3 times a week for 11 mo. During the 12th mo the HGH dose was changed to 1 U (National Pituitary Agency) nightly. Height and weight were recorded biweekly. At 11¾ mo the patient was readmitted to the metabolic research ward. After 3 days' equilibration with the metabolic diet, a 42 day study was done as follows: (*a*) 7 days at 1 U daily; (*b*) 7 days' control period (no HGH treatment); (*c*) 7 days at dose B; (*d*) 7 days' control period; (*e*) 7 days at dose C; (*f*) 7 days' control period.

(*c*) *After 3 mo at home without HGH* (*exp. 3*). The patient was now readmitted for another 35 days' metabolic balance study identical to exp. 1 above.

HGH antibodies were determined radioimmunologically by the method of Roth, Glick, Yalow, and Berson (3), as modified by Illig (4).

RESULTS

All six children showed the same pattern of findings. Before long-term treatment began, N, P, Na, and K balances were close to zero. Dose B caused positive balance of all four elements with associated weight gain; dose C had a similar but larger effect (Fig. 1; Table II). When dose C was stopped, the patients' balances returned to zero within 48 h.

During the entire year of treatment the rate of growth accelerated (Fig. 2). But the rate was highest (15–25 cm/yr) during the first 3–6 mo and then declined (0–8 cm/yr). In all six cases, the inflection in the growth curve occurred between 3 and 7 mo.

After 12 mo of treatment the balances were not detectably different from zero (Fig. 1). When HGH was withdrawn, all six cases immediately (within 24 h) developed negative balances of N, P, Na, and K, with associated weight loss. This catabolic behavior continued

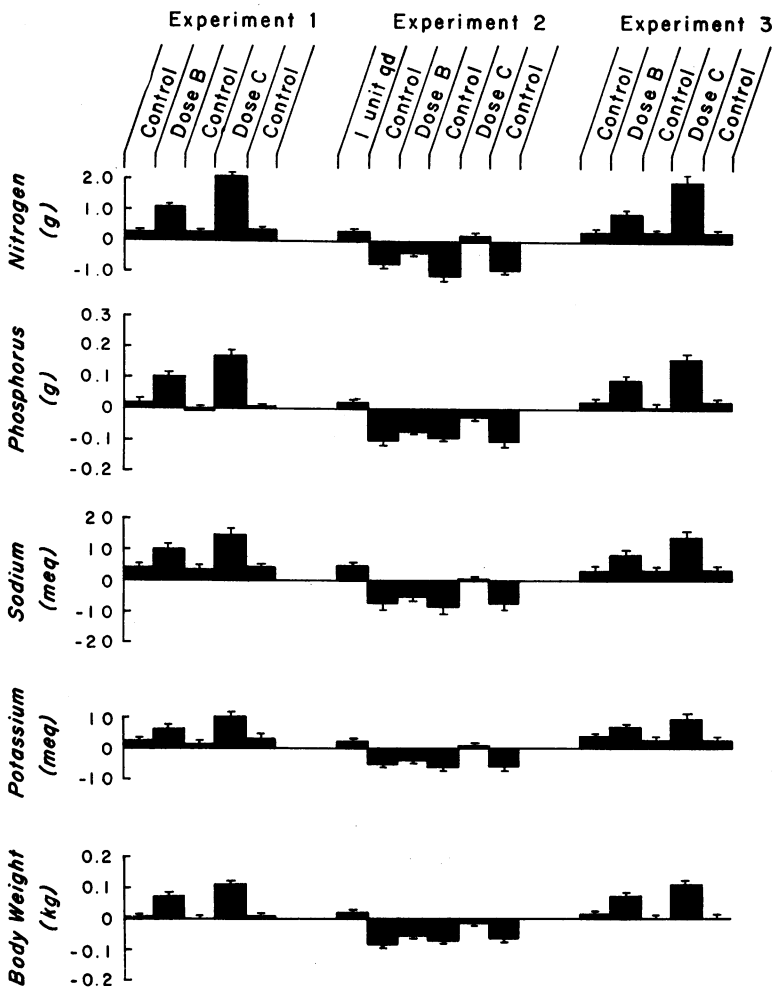

FIGURE 1 Average elemental balances and BW changes of the six subjects during experiments 1, 2, and 3. SE of each mean is also shown.

for 7 days, at which time dose B was begun. Under the influence of dose B balances became less negative. Dose C returned the balances to zero or to slightly positive values (Fig. 1). When dose C was stopped, the patients immediately returned to negative balances. The magnitude of the increments in balances and in BW produced by doses B and C were only 20–60% as great as 12 mo earlier (Table II).

The results 3 mo later resembled those before long-term treatment (Fig. 1). Balances during the first control period were close to zero. Doses B and C caused anabolic responses of the same magnitude as before the year of HGH replacement (Table II). When dose C was stopped, the patients returned to approximately zero balance; net loss of elements did not occur.

Antibodies to HGH were not detectable in the plasma of any patient at the beginning of the 1-yr course of treatment, nor at the end of the year in patients 1, 2, 4, and 6. In cases 3 and 5, respectively, antibody titers of 1:20 and 1:100, and binding capacities of 0.008 and 0.056 mg HGH/liter plasma, were present at 12 mo. 3 mo later, these values were: case 3, titer 1:20 and binding capacity 0.015 mg HGH/liter; case 5, titer 1:100 and binding capacity 0.035 mg HGH/liter.

DISCUSSION

The GH-deficient child responds to a 7 day course of dose B or C of HGH with a vigorous anabolic reaction (1). In such children, continuous treatment with dose B to C of HGH leads within 1 mo to acceleration of linear growth (5–8) (Fig. 2). Around 6 mo, the accelerated rate of growth declines and persists at the lower level during the next 6 mo (9) (Fig. 2). At this time the anabolic responses to dose B and C are only 20–60%

TABLE II

Comparison of Elemental Balances and of Responses to Doses B and C of HGH in GH-Deficient Children before, during, and after 1 yr Treatment with HGH.

	ΔN	ΔP	ΔNa	ΔK	ΔBW
(A) Response to dose B before 1 yr treatment (exp. 1)	+0.9	+0.08	+5.6	+4.0	+0.07
	±0.13	±0.009	±0.75	±0.67	±0.008
(B) Response to dose B at end of 1 yr treatment (exp. 2)	+0.3	+0.02	+2.1	+1.4	+0.025
	±0.009	±0.004	±0.4	±0.28	±0.003
(C) Response to dose B 3 mo after 1 yr treatment (exp. 3)	+0.6	+0.07	+4.9	+2.5	+0.05
	±0.09	±0.009	±0.46	±0.27	±0.006
P for (B) vs. (A)	<0.001	<0.001	<0.005	<0.02	<0.001
P for (C) vs. (A)	NS	NS	NS	NS	NS
P for (C) vs. (B)	<0.01	<0.001	<0.001	<0.05	<0.005
(A) Response to dose C before 1 yr treatment (exp. 1)	+1.8	+0.17	+11.0	+8.5	+0.11
	±0.14	±0.02	±1.9	±0.79	±0.01
(B) Response to dose C at end of 1 yr treatment (exp. 2)	+1.1	+0.08	+8.0	+7.6	+0.06
	±0.13	±0.008	±0.75	±0.67	±0.007
(C) Response to dose C 3 mo after 1 yr treatment (exp. 3)	+1.6	+0.16	+10.3	+7.0	+0.11
	±0.09	±0.009	±1.6	±0.83	±0.008
P for (B) vs. (A)	<0.005	<0.005	NS	NS	<0.005
P for (C) vs. (A)	NS	NS	NS	NS	NS
P for (C) vs. (B)	<0.01	<0.001	NS	NS	<0.001

Values represent average ±SE for six patients. NS signifies $P > 0.05$.

those which were observed before long-term treatment (Table II).[2]

The present experiments confirm the impression of Henneman, Forbes, Moldawer, Dempsey, and Carroll (10) in 1960 that the anabolic response to HGH gradually diminishes during continuous treatment. These investigators treated six hypopituitary patients with HGH at about 1–3 times dose C for 5–7 wk; the resulting positive elemental balances waned after 3–4 wk of treatment. Responsiveness was restored by a rest period of 12 days.

The decline in responsiveness to exogenous HGH could result from development of neutralizing antibodies, or from loss of responsiveness to the hormone by the peripheral tissues. The first possibility is excluded by the data on plasma antibody levels. Responsiveness to HGH diminished in a similar manner in the four children who did not produce antibodies, as in the two who did develop an antibody response. Furthermore, in the latter two patients, 3 mo interruption of HGH treatment led to an increase in anabolic responsiveness to the hormone without change in antibody titer.

At what point during the 1 yr treatment does the peripheral responsiveness to HGH decline? Further experiments at various times during the year will be necessary to answer this question. It is reasonable to speculate that the inflection in the growth curve at 3–6 mo may be a manifestation of such a loss of responsiveness. This inference is supported by the fact that non-GH-deficient children, in whom dose B–C of HGH has little effect on elemental balances (1), show little or no accelera-

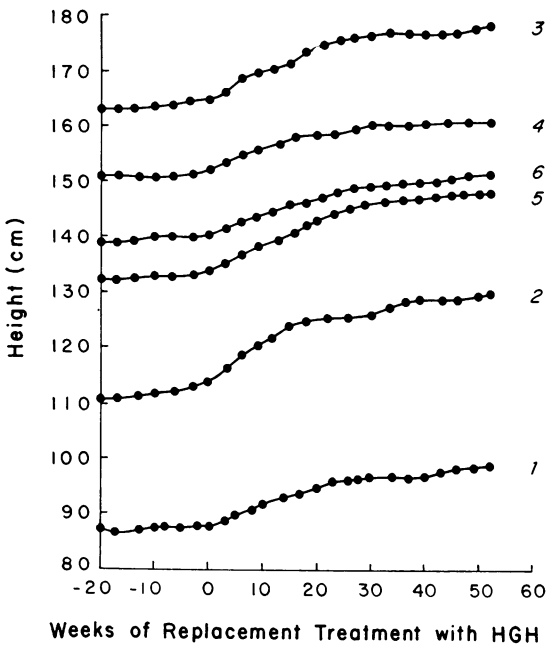

FIGURE 2 Growth curves before and during treatment of six GH-deficient children with HGH for 1 yr.

[2] During the control period of exps. 1 and 3, the elemental balances were zero or slightly positive (Fig. 1). During the control period of exp. 2, the balances were negative. A comparison of the magnitude of the anabolic responses in exps. 1, 2, and 3 can be only approximate, because of this difference in the base line for the measurement of the responses in exp. 2 vs. exps. 1 and 3.

tion of linear growth in response to long-term treatment with these doses (11, 12). When the precise timing and relationships of these two events, change in slope of the growth curve and loss of anabolic responsiveness to HGH, have been defined, it may be possible to design a new schedule of intermittent HGH treatment which will maintain a higher average rate of growth during long-term treatment.

Previous studies, comparing GH-deficient ("hyposomatotropic") and normal ("eusomatotropic") children showed the former to be more responsive than the latter to the anabolic actions of HGH (1, 13). Further manifestation of this phenomenon is now provided by comparisons within the same individual. The six cases studied here were hyposomatotropic at the time of exps. 1 and 3, and relatively eusomatotropic at the time of exp. 2 when they had received replacement treatment for 1 yr. In each individual, the anabolic responses in exp. 2 were 40–80% smaller than those in exps. 1 and 3.

While the present study provides new evidence for the relative resistance of individuals not deficient in endogenous GH to the anabolic effects of exogenous HGH, it also indicates indirectly that these individuals are sensitive to the metabolic effects of their endogenous GH. The question of whether endogenous GH is an important regulator of metabolic events besides growth is an old one. Ordinarily such a problem could be investigated by ablating the hormone and observing the results. This approach is complicated, in the case of GH, by the presence of several other metabolically active hormones in the gland of origin. Russell (14) observed acute negative N balance immediately after hypophysectomy in the rat and speculated that this might signify a role of GH in the regulation of N metabolism of the rodent. The present experiments, involving correction of endogenous GH deficiency for 1 yr in man followed by abrupt withdrawal of the hormone, provide a more specific approach to the problem than total hypophysectomy, since adrenal, thyroid, and neurohypophyseal functions remained normal when GH was selectively and nontraumatically withdrawn. Our results confirm in man the conclusion of Russell in the rodent, in that deletion of GH in human subjects caused acute negative N balance. This was accompanied, moreover, by acute negative balance of other intracellular (K and P) and extracellular (Na) elements and by loss of body weight. From the ratios of the negative balances of N, P, Na, and K, it can be calculated (15) that the loss of body weight represented about 50% intracellular protoplasm and 50% extracellular fluid. These observations, although made in GH-deficient subjects during and after replacement treatment with exogenous GH, suggest that in normal individuals the endogenous hormone is indeed involved in the continuing regulation of protein and mineral metabolism.

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