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Whole Body Nitrogen Kinetics and Their Relationship to Growth in Short Children Treated with Recombinant Human Growth Hormone

DAVID P. DEMPSHER, DENNIS M. BIER, SHERIDA E. TOLLEFSEN, PETER S. ROTWEIN, WILLIAM H. DAUGHADAY, MARY CATHERINE JENSEN, JOHN P. GALGANI, ELLEN HEATH-MONNIG, AND BAKULA TRIVEDI

Department of Pediatrics and Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT. We studied the effects of growth hormone on retention of ^{15}N-labeled amino acids in 34 short, prepubertal, growth hormone-sufficient children and three growth hormone-deficient subjects. All 34 non-growth hormone-deficient children had apparently normal circulating growth hormone molecules and no mutations were detected in the growth hormone or IGF-I genes of any subjects. Fibroblasts from 34 children responded normally when challenged with recombinant human IGF-I. During the last 72 h of a 4-d challenge with recombinant human growth hormone (16 μg/kg body wt), retention of a mixed ^{15}N-amino acid dose varied between 5.7 and 50.5%. Whole body protein synthesis, breakdown, and net anabolism calculated from the ^{15}N kinetics were all increased by the acute growth hormone challenge. However, no routine clinical feature or laboratory determination correlated with the nitrogen retention response. After subsequent treatment (75 μg/kg three times a week) with recombinant human growth hormone for 1 y, there was a significant increase in height velocity, but this increase was not related significantly to pretreatment variables other than inversely to pretreatment height velocity. There was a significant ($p = 0.03$) correlation between the change in height velocity Z score and the degree of nitrogen retention to acute challenge with growth hormone, but this correlation was too weak ($r = 0.37$) to be of practical value in predicting the treatment growth response in an individual child. (*Pediatr Res* 28: 394–400, 1990)

Abbreviations

rhGH, recombinant human growth hormone
ANOVA, analysis of variance
IRMA, immunoradiometric assay

It is now abundantly clear that some short children whose growth hormone secretion is regarded as normal after provocative stimulation will augment their height velocity significantly when treated with exogenous growth hormone (1–9). One implication of these data is that these children have dysfunctional regulation of growth hormone secretion under physiologic conditions that is masked by pharmacologic provocation. Thus, there is an increasing need for alternative ways of assessing the physiologic adequacy of a child's growth hormone secretion.

Protein accretion is the sine qua non for growth, and pituitary extracts rich in growth hormone have been known for more than half a century to produce nitrogen retention (10–12). For this reason, before the availability of a growth hormone RIA, improvement in nitrogen balance after growth hormone administration was used routinely for diagnosis of growth hormone deficiency and for assessment of therapeutic response (13–22). Even after the measurement of plasma growth hormone concentration became practical, testing the subject's nitrogen retention response to growth hormone administration remained a clinical and investigative tool (23–38).

^{15}N-labeled amino acids have been used extensively to measure total body nitrogen kinetics in adults and children (39). Unlike classical nitrogen balance studies, studies using ^{15}N-amino acids can be completed in days or, under certain circumstances (40), hours. In addition, with appropriate models, the tracer data allow calculation of whole body protein flux, synthesis, and catabolism, rather than simply net nitrogen balance. Small numbers of short children with growth hormone deficiency have been studied with ^{15}N techniques in the past (41, 42). More recently, Zachmann *et al.* (43) used the ^{15}N-retention test to show the biologic equivalency of recombinant and pituitary-derived hGH preparations. Furthermore, Richter *et al.* (44) reported that the degree of retention of a tracer dose of [^{15}N]glycine induced by growth hormone administration allowed differentiation of children with normal growth hormone secretion from those with complete or partial growth hormone deficiency.

For the above reasons, we postulated that a functional test of growth hormone effect on nitrogen kinetics may provide an index of growth hormone adequacy in short children and, perhaps, predict the response to long-term growth hormone therapy. The results of the test of this hypothesis are described below. A preliminary report of this study (45) and the IGF-I gene characterization (46) have appeared previously.

MATERIALS AND METHODS

Subjects. Thirty-seven prepubertal children between the ages of 6 and 14 y and more than two SD below the mean height for age were recruited from the Washington University Pediatric Endocrinology Clinic. Their characteristics are summarized in Table 1. Chronic underlying medical illnesses were excluded by history and appropriate conventional diagnostic testing when indicated. All had normal thyroid function. All eight girls had normal karyotypes. The subjects' and parents' heights were measured by trained personnel using a wall-mounted Harpenden stadiometer. Similarly obtained pretreatment height velocity measurements were recorded for a minimum period of 1 y before

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Reprint requests: Dennis M. Bier, M.D., Metabolism Division, Box 8127, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110.
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formal enrollment in the study described below. Informed consent was obtained from all families according to procedures approved by the Washington University Human Studies Committee.

Analytical methods. Plasma growth hormone concentrations were quantified by conventional polyclonal RIA, by IM-9 lymphocyte (47) or human liver radioreceptor assay (48), and by two-site monoclonal IRMA (Hybridtech, San Diego, CA). Serum growth hormone binding protein (49) and circulating IGF-I concentration (50) were measured as described previously. Osteocalcin was measured by specific RIA using a commercial antibody (Incstar Corp., Stillwater, MN).

In all subjects, high mol wt chromosomal DNA was isolated from nuclei of peripheral blood cells by standard methods (51). DNA (10 μg) was digested individually with restriction endonucleases *Bam*HI, *Eco*RI, and *Hin*dIII, followed by electrophoresis and transfer to nitrocellulose or nylon membranes as described (52). Prehybridization of the membranes, hybridization to a ^{32}P-labeled (53) hGH cDNA probe (Genentech, Inc., South San Francisco, CA) and posthybridization washes followed the protocol of Wahl *et al.* (54). Hybridization bands were detected by autoradiography at −80°C using DuPont Lightening Plus intensifying screens and Kodak XAR5 x-ray film. In addition, each child's IGF-I gene was examined as described (46) and the results have been reported in detail earlier (46). A 2-mm punch biopsy specimen of skin was obtained from 34 of the 37 children for assessment of cultured fibroblast aminoisobutyric acid uptake response to recombinant human IGF-I according to methods detailed previously (55).

Plasma glucose was determined by a glucose oxidase method using the Beckman glucose analyzer, and plasma insulin (56) and urinary C-peptide (57) were measured by polyclonal RIA as described previously. Urinary creatinine, urea, and ammonia were measured by standard methods (40) using commercial kits (Sigma Diagnostics, St. Louis, MO) and urinary total nitrogen was quantified by chemiluminescence using a nitrogen analyzer (Antek Instruments, Houston, TX). ^{15}N enrichment in urinary total nitrogen, urea, and ammonia were determined by dual-inlet, dual-collector isotope ratio mass spectrometry according to classical methods (40, 58, 59).

Procedural methods. Before participation in the kinetic study and treatment protocol below, analyses of growth hormone binding protein and the growth hormone and IGF-I genes were carried out in each subject as described above. Plasma growth hormone responses to oral clonidine (5 μg/kg) and i.v. insulin (0.1 U/kg) provocation were measured in the overnight postabsorptive state. The circulating growth hormone concentration was quantified by polyclonal RIA in all samples. In each provocative test, the two plasma samples in which growth hormone concentration was the highest when measured by polyclonal RIA were remeasured by monoclonal IRMA or by IM-9 or human liver radioreceptor assays as described above.

Nitrogen kinetics. Subsequently, each child was admitted to the Washington University Pediatric Clinical Research Center for 9 d to study the acute effects of growth hormone administration on nitrogen kinetics. According to a prescription provided by the research dietitian after a diet history, each subject consumed an isocaloric diet containing 1 g of protein per kg body wt daily for the week before admission. This procedure was intended to minimize changes in dietary protein intake on admission, when a fully monitored diet containing the same dietary energy and protein intakes was consumed for the entire 9-d hospitalization.

During the hospitalization, blood was drawn each morning in the postabsorptive state and assayed for glucose, insulin, IGF-I, and, on the 4th and 9th days, for osteocalcin as described above. All urine was collected, acidified, and frozen in 8-h aliquots for later analysis of urinary nitrogen end-products and their ^{15}N enrichments as noted above. On the 2nd and 6th hospital days, a dose of mixed ^{15}N-labeled amino acids (1 mg ^{15}N/kg body wt) was administered orally with breakfast. The composition of the mixture (mg amino acid/kg body wt) was as follows: leucine 1.007; valine 0.901; methionine 0.306; phenylalanine 0.806; lysine 1.007; alanine 0.606; aspartic acid 0.701; glutamic acid 0.806; glycine 1.007; serine 0.507; and tyrosine 0.401.

From the morning of the 5th day until discharge rhGH (Humatrope, Eli Lilly Co., Indianapolis, IN) was injected s.c. every 12 h at the dose of 16 μg/kg body wt. Urine samples collected for 72 h as above for nitrogen content and isotopic enrichment on the 2nd, 3rd, and 4th, and on the 6th, 7th, and 8th hospital days will be referred to subsequently as the pre- and post-growth hormone samples, respectively.

Growth response. On discharge from the hospital, each child was treated with rhGH (Humatrope) at the dose of 75 μg/kg body wt given three times weekly by s.c. injection. At three monthly intervals over the ensuing 6 to 12 mo, each child was reexamined and his/her height measured with a Harpenden stadiometer. If a child failed to exceed his/her pretreatment height velocity by at least 2 cm/y at the 6-mo visit, growth hormone therapy was discontinued. Final examination and height measurement were performed in the remaining "responders" at the 12-mo visit.

Calculations. Total ^{15}N in each urine sample was calculated as the product of the ^{15}N enrichment and the total nitrogen content. Cumulative ^{15}N excretion was the sum of the total urinary ^{15}N excretion in the pre- and post-growth hormone samples obtained over the 72 h after administration of the tracer dose. Similarly, cumulative 72-h [^{15}N]urea excretion was calculated from the urinary [^{15}N]urea enrichment and the urinary urea nitrogen content in each sample. Whole body nitrogen and protein kinetic parameters were calculated using established expressions according to the classical, single nitrogen pool model (39).

Values are reported as mean ± SD. Overall statistical comparisons were made by ANOVA with *t* test for paired analyses used

Table 1. *Clinical characteristics of patient population**

Parameter	Before GH treatment (mean ± SD)	After 1 y GH treatment (mean ± SD)
n	37	32
CA (y)	8.7 ± 2.6	9.7 ± 2.6
BA (y)	6.3 ± 2.3	7.5 ± 2.5
Wt (kg)	20.2 ± 5.7	24.6 ± 7.1
Ht (cm)	113.4 ± 13.5	122.3 ± 13.6
Ht (Z score)	−3.0 ± 0.7	−2.4 ± 0.7†
Ht velocity (cm/y)	4.7 ± 1.2	8.3 ± 1.6†
Ht velocity (Z score for CA)	−1.4 ± 1.4	3.1 ± 2.7†
Ht velocity (Z score for BA)	−1.8 ± 1.1	2.2 ± 2.0†
RWT predicted ht (cm)	162 ± 5.6	164 ± 6.4
RWT predicted ht (Z score)	−3.0 ± 0.7	−2.4 ± 0.7†
Mother's ht (cm)	157.4 ± 5.2	
Mother's ht (Z score)	−1.1 ± 0.9	
Father's ht (cm)	173 ± 7.5	
Father's ht (Z score)	−0.5 ± 1.1	

* Abbreviations: CA, chronologic age; BA, bone age; RWT, Roche-Wainer-Thissen.

† $p < 0.001$ compared with pretreatment value. CA, BA, wt, and absolute ht were not tested for significance because their increase was dictated by study design and duration.

when appropriate. Correlations were calculated by univariate linear regression.

RESULTS

Clinical characteristics. The clinical characteristics of the subjects are shown in Table 1. By design, all were prepubertal with a mean age of 8.7 ± 2.7 (SD) y and height Z score of −3.0 ± 0.7. On the average, bone age was delayed by slightly more than 2 y and the group, as a whole, had parents whose average heights were shorter than the 50th percentile height for normally sized adults.

Plasma growth hormone. Twenty-six of the 37 children had a peak plasma growth hormone response of greater than 10 ng/mL to insulin and/or clonidine provocation. Eight had a peak plasma growth hormone value greater than 7 but less than 10 ng/mL to one test but less than 7 ng/mL to the other test. Three of these eight children had plasma growth hormone values of greater than 10 ng/mL measured during sleep. Three additional children had peak plasma growth hormone responses less than 7 ng/mL to both clonidine and insulin challenge and were classified as growth hormone deficient. None of the latter three had hormonal evidence of additional TSH or ACTH deficiency. Because the subsequent responses of the growth hormone-deficient children to acute and chronic growth hormone supplementation were indistinguishable from those of the remaining 34 subjects, the results for the growth hormone-deficient children will be included with those of the study group as a whole but identified individually when appropriate.

Of the 34 non-growth hormone-deficient subjects, none had a clearly abnormal circulating growth hormone molecule estimated from the ratio of radioreceptor or of monoclonal IRMA assayable growth hormone to the value obtained using conventional polyclonal RIA (45). Thus, the average radioreceptor assay/RIA and IRMA/RIA plasma growth hormone ratios in the selected samples described earlier were 0.97 ± 0.28 (SD) and 0.88 ± 0.22, respectively, with no value below 0.5, the 5th percentile ratio found in subjects of normal height. Similarly, no subject had absent serum growth hormone binding protein (47).

Growth hormone and IGF-I genes. The growth hormone gene locus was analyzed in all 37 subjects and in 10 controls of normal stature by hybridization of a ^{32}P-labeled hGH cDNA to chromosomal DNA digested with three restriction enzymes as described in Methods. A representative autoradiograph of *Bam*HI-digested DNA from eight subjects is illustrated in Figure 1. As indicated, all five genes within the 48-kb cluster are visualized and appear normal in length (53). A similar normal pattern was observed in all subjects with each restriction enzyme. Additionally, the present group of 37 children was included in a previous report of 61 short children who were evaluated for mutations in the IGF-I gene (46). Analysis of chromosomal DNA from these children failed to reveal any variation in the IGF-I gene except for a *Hin*dIII site polymorphism present in normally sized individuals as well (46).

Fibroblast responsiveness to IGF-I. All 34 fibroblast cell lines tested for aminoisobutyric uptake response to challenge with recombinant human IGF-I (55) responded normally with a mean ED_{50} value of 2.41 ± 0.71 ng/mL and a 2.50 ± 0.40-fold maximal stimulation of uptake (55).

Nitrogen kinetic response. Daily total urinary nitrogen excretion declined slightly but significantly ($p < 0.05$ by ANOVA) between the 1st and 2nd days of hospitalization (Fig. 2) but remained unchanged from the 2nd through the 4th days. Growth hormone injections, begun on the 5th hospital day, induced a second decline in urinary total nitrogen, which achieved a new constant level on d 6 through 8 (Fig. 2). There was no change in the excretion ratios of urea or ammonia to total nitrogen, and creatinine excretion remained unchanged throughout the hospitalization (data not shown).

The total cumulative excretion of ^{15}N after the first tracer dose (72 h pre-growth hormone urines) was compared with the cumulative ^{15}N excretion of the second tracer dose during 72 h of s.c. growth hormone administration at the dose of 16 g per kg body wt every 12 h (post-growth hormone urines). Before the administration of growth hormone, cumulative ^{15}N excretion averaged 20.3% of the dose. During acute supplementation with

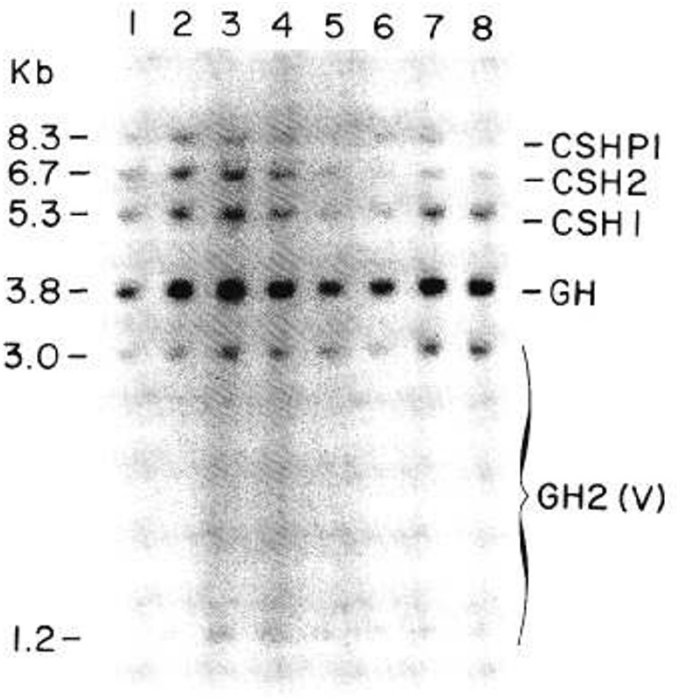

Fig. 1. Analysis of the growth hormone locus in chromosomal DNA from eight short subjects. DNA was digested with *Bam*HI; electrophoresis, transfer to a nylon membrane, and hybridization of ^{32}P-hGH cDNA were performed as described in Methods. Fragment sizes (kb) and the gene contained within each detected fragment are indicated.

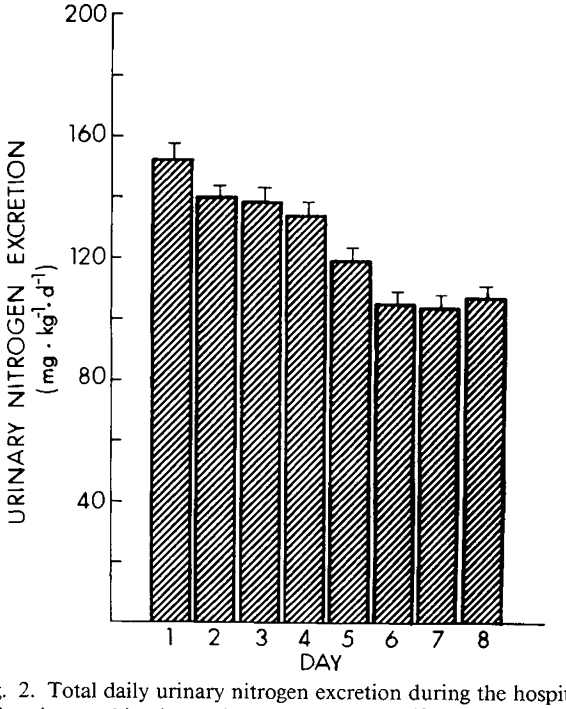

Fig. 2. Total daily urinary nitrogen excretion during the hospitalization for nitrogen kinetic studies. The two mixed ^{15}N amino acid tracer doses were administered with breakfast on the mornings of d 2 and 6. Subcutaneous administration of rhGH at 12-h intervals was begun on the morning of d 5 (see text).

exogenous growth hormone, ^{15}N excretion declined an average of 31 ± 10%, but the change varied considerably between subjects, ranging from a 5.7 to a 50.5% decrease (Fig. 3). The three growth hormone-deficient children diminished their ^{15}N excretions by 31.5, 31.8, and 50.5%. ANOVA showed no statistically significant relationships between the subjects' clinical characteristics, bone age delay, and IGF-I level and the degree of nitrogen retention after growth hormone administration.

Figure 4 shows the results of whole body protein kinetic parameters calculated from the urinary total nitrogen and total ^{15}N-excretion data as described (39). Before growth hormone treatment, whole body protein turnover, synthesis, and catabolism were 4.38 ± 0.56, 3.52 ± 0.60, and 3.38 ± 0.5 $g \cdot kg^{-1} \cdot d^{-1}$, respectively. Growth hormone challenge increased body protein turnover by enhancing both protein synthesis and protein breakdown (Fig. 4), but because synthesis increased more than breakdown, net body protein accretion (synthesis minus catabolism) increased by more than 200% from 0.14 ± 0.03 to 0.35 ± 0.02 $g \cdot kg^{-1} \cdot d^{-1}$ ($p < 0.001$).

Growth hormone challenge at 16 μg/kg every 12 h caused an increase in the postabsorptive serum IGF-I level from a pregrowth hormone value of 0.56 ± 0.48 to 0.97 ± 0.56 U/mL by the morning of the 4th day of treatment, significant at the $p < 0.05$ level by ANOVA. By similar analysis, there was no relationship between any of the whole body protein kinetic indices derived from the ^{15}N-tracer data and the change in circulating IGF-I. The acute 4-d treatment with rhGH caused no change in circulating growth hormone binding protein and no significant change was observed in fasting plasma glucose or insulin values (Fig. 5*a*) during the period of growth hormone challenge. However, daily urinary C-peptide excretion (Fig. 5*b*) rose significantly ($p < 0.05$) within 24 h of growth hormone administration and stayed elevated throughout the remaining period of growth hormone treatment. Serum osteocalcin was 11.8 ± 2.0 ng/mL before growth hormone supplementation and remained unchanged after 4 d of rhGH injections. In no individual was there a substantial change in serum osteocalcin.

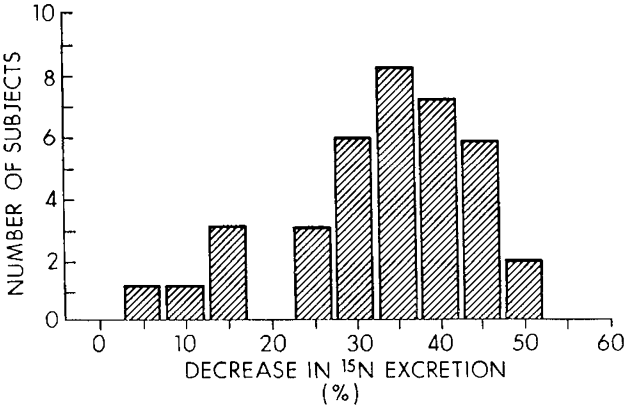

Fig. 3. Distribution of the percent change in excretion of the mixed ^{15}N amino acid tracer dose by the study subjects during 72 h of growth hormone treatment.

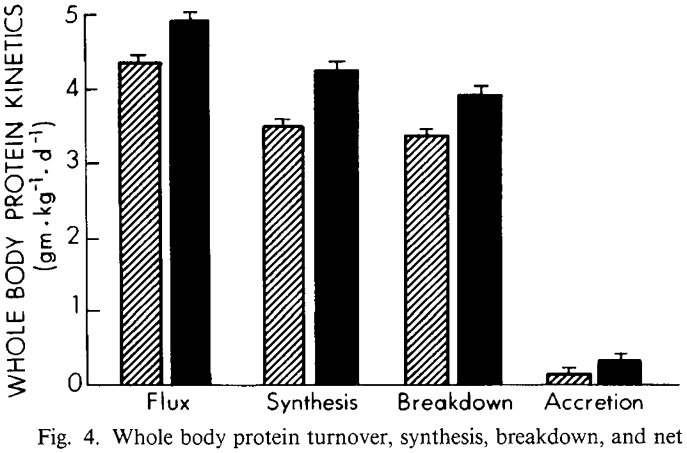

Fig. 4. Whole body protein turnover, synthesis, breakdown, and net protein accretion (synthesis minus breakdown) in the study subjects before and during 72 h of growth hormone treatment.

Growth response. Of the 37 subjects who had their nitrogen kinetic responses measured, one dropped out of the study before the 3-mo visit and one voluntarily discontinued follow-up at 9 mo of therapy. Data from these two subjects are excluded from discussion of long-term response to growth hormone treatment. At the 6-mo treatment visit, growth hormone treatment of three children was discontinued because their height velocities had not increased by more than 2 cm/y above their pretreatment height velocity values. The complement of this observation is that 33 of the 36 individuals who were treated with growth hormone for 6 mo grew at an accelerated rate. Of the 32 children who completed a full year of treatment, two girls developed breast buds by 1 y of therapy. Their data are included in subsequent analyses because pubertal progression was minimal and exclusion of their data had no effect on the statistical analyses that follow. No adverse effects were detected during the prolonged growth hormone treatment. We observed no significant changes in postabsorptive blood glucose, cholesterol, or triglyceride values measured by routine clinical chemistry assays. There was no significant rise in antibody titers to *Escherichia coli* proteins or to growth hormone as measured by the standardized assays at the Eli Lilly Company.

Table 1 shows the growth parameters for the 32 children at the end of a year's treatment with rhGH at the dose of 75 μg/kg body wt administered s.c. three times a week. Despite the fact that incremental annualized height velocity declined to less than 2.0 cm/y over pretreatment height velocity in an additional six children during the 2nd 6 mo of growth hormone treatment, the measured yearly posttreatment height velocity of the group as a whole was still significantly elevated at 8.3 ± 1.6 cm/y ($p < 0.001$), a comparison maintained as well for height velocity Z scores expressed for both chronologic and bone ages (Table 1). The three growth hormone-deficient subjects increased their absolute height velocities by 2.6, 6.3, and 9.2 cm/y and their height velocity Z scores by 3.8, 8.7, and 12.8.

The average height of the subject group increased from 113.4 to 122.3 cm, representing a significant ($p < 0.05$) mean change in height Z score of +0.64 ± 0.33. The ratio of the change in bone age to the change in chronologic age averaged 1.3 ± 0.8, a value not statistically different from 1.0 by *t* test. Predicted adult height increased, but not significantly (Table 1). Neither pretreatment IGF-I nor the acute change in IGF-I to growth hormone challenge predicted the long-term growth response. Only a low pretreatment height velocity correlated significantly with the change in annualized growth rate at 1 y of treatment ($r = -0.6$, $p < 0.001$).

Neither the measured growth rates nor the increments in height velocity above pretreatment values (when expressed in absolute terms of cm/y) at 3, 6, or 12 mo of therapy were correlated with any of the indices of protein dynamics derived from the ^{15}N data, including the percent change in ^{15}N excretion itself. Figure 6 shows the relationships between the change in ^{15}N retention induced by the acute growth hormone challenge and the increment above pretreatment height velocity measured after 1 y of treatment with rhGH. When the change in height velocity measured after 1 y of rhGH treatment was expressed as a Z score to normalize for age-related differences in growth rate, there was a weak but significant correlation ($r = 0.37$, $p = 0.03$) with the change in ^{15}N retention to the acute growth hormone challenge (Fig. 6). Obviously, for an individual subject, this relationship was too weak to be predictive of height velocity response to growth hormone treatment.

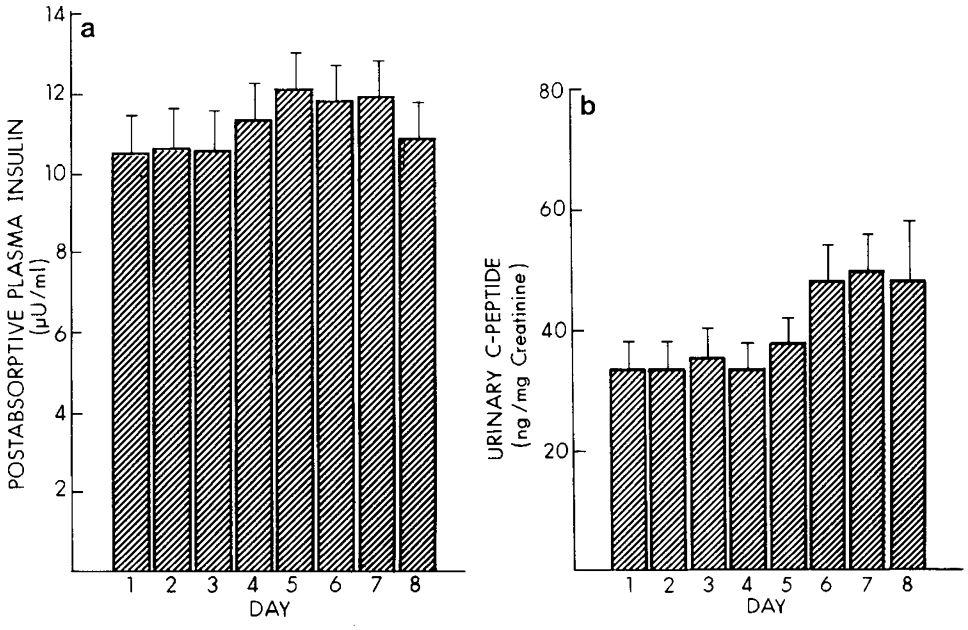

Fig. 5. *a*, Postabsorptive plasma insulin values during the hospitalization for nitrogen kinetic studies. *b*, Twenty-four h urinary C-peptide excretion over the same period. Growth hormone administration was begun on the morning of d 5 (see text).

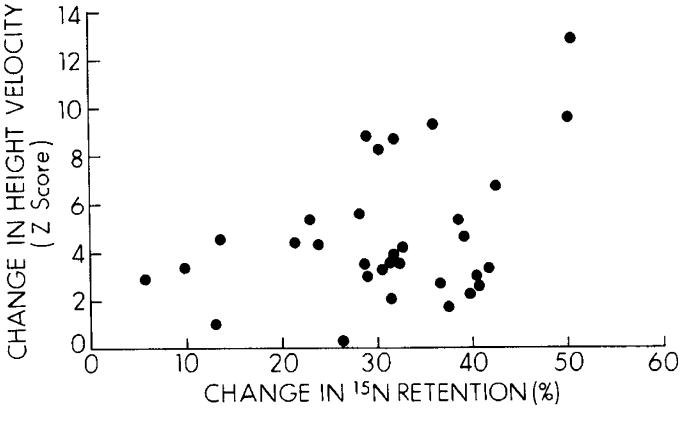

Fig. 6. Relationship between the change in nitrogen retention to an acute challenge with growth hormone and the change in ht velocity at the end of 1 y of treatment with rhGH, expressed as change in height velocity Z score for age. This relationship is defined by the equation $y = 0.15x \pm 1.4$ ($r = 0.37$) and is significant at the $p = 0.03$ level.

DISCUSSION

In our study, the acute metabolic response to growth hormone was tested in a group of 34 short children whose growth hormone secretion was normal after challenge with conventional provocative stimuli and three children who met the classical criteria of growth hormone deficiency. Subsequently, all were treated with rhGH for 6 mo to 1 yr. Thirty-two children completed a year's therapy.

The study population was well characterized. In addition to conventional clinical assessment and routine measurements of bone age, growth hormone secretion, and circulating IGF-I, the subjects' growth hormone and IGF-I genes (46) were shown to be normal. There was no gross structural abnormality of the circulating growth hormone molecule as probed by radioreceptor and monoclonal antibody assays (47, 48). No child had absent growth hormone receptors as assessed by assaying circulating growth hormone binding protein levels (49). Each subject's cultured fibroblasts were normally sensitive to IGF-I with regard to uptake of aminoisobutyric acid (55).

As in other reports (1–9), we showed that a significant percentage of children who had apparently normal growth hormone secretion will augment their height velocity significantly when treated with exogenous growth hormone. Thirty-three of the 36 subjects treated for 6 mo grew at a height velocity 2.0 cm/y or more greater than their pretreatment rate. Of the 32 subjects completing a year of growth hormone treatment, 29 had grown a measured annual height velocity of 2.0 cm or more faster than the pretreatment rate and half grew 3.0 cm or more faster than their pretreatment height velocity. On average, the children's height velocity was 77% faster after 1 y of treatment with rhGH, 8.3 ± 1.6 *versus* 4.7 ± 1.2 cm/y (Table 1). These results are similar to previous studies in other groups (7–9).

Consistent with the reports of others (7, 8), and except for the inverse correlation with the child's pretreatment height velocity also shown by Lin *et al.* (7), we were unable to find any correlation between anthropometric parameters, bone age, or circulating growth hormone levels achieved after provocative testing and subsequent growth response to treatment. In contrast to the largest study reported to date (8), we were also unable to find a relationship between the child's pretreatment serum IGF-I concentration and subsequent growth response to treatment or to calculate significantly improved prediction of final adult height. Others have also failed to find the former relationship (7) or the latter apparent improvement (9). Nor did we find a relationship between the acute changes in serum IGF-I and subsequent growth, consistent with the data of Gertner *et al.* (4).

In an attempt to provide a more physiologic predictor of growth response to chronic growth hormone treatment, we assessed the child's nitrogen retention response to an acute challenge with growth hormone and quantified whole body nitrogen and protein kinetics using ^{15}N-labeled amino acids. The former approach is not new (13–38) but the latter method has been little used (41–44). Its potential advantages are several. In particular, however, is the advantage that addition of the tracer allows one to distinguish between an increase in protein synthesis and a decrease in protein breakdown as the mechanism of reduced nitrogen excretion after growth hormone administration. For this purpose, we used a mixture of ^{15}N-labeled essential and nonessential amino acids rather than the more commonly used [^{15}N]glycine in an attempt to label the nitrogen pool more uniformly. Because glycine constitutes about ⅓ the composition of collagen, collagen itself represents about 20–25% of total body protein, and growth hormone's effects on collagen (60) might

not reflect its actions on body protein synthesis as a whole, we elected to avoid potential bias introduced by using this amino acid singly as a tracer.

On the average, growth hormone enhanced ^{15}N retention by 31%, but the variability in the nitrogen response to rhGH was striking (range 5.7–50.5%) considering that the subjects were on the same reasonably constant dietary protein intake for 8 d before the first tracer study. Richter *et al.* (44) found a similar variability using [^{15}N]glycine tracer. However, in contrast to Richter *et al.* (44), we were unable to find a relationship between a child's growth hormone status determined with provocative testing and the child's ^{15}N retention after growth hormone challenge. Thus, for example, two of the three children who failed both growth hormone provocative tests had ^{15}N retention responses virtually identical to the mean value. This result is also decidedly different from several earlier studies (23, 25, 27, 29, 30, 32, 61) where children with growth hormone deficiency had distinctly greater reductions in urinary nitrogen excretion than children who were not deficient in this hormone.

The ^{15}N tracer allowed us to show for the first time in a large, well-defined group of short children that the growth hormone-induced decrease in nitrogen excretion is not the result of decreased protein breakdown. Body protein breakdown, in fact, increased, but less so than synthesis. Thus, there was a net anabolic effect. Presumably, the increase in body protein breakdown reflects the necessary remolding of body structural proteins necessary for growth. These data complement the recent report of Dahms *et al.* (62) who showed, under similar circumstances in a group of growth hormone-deficient children, that growth hormone administration produced a decrease in urea synthesis. Because the latter authors concluded that the decline in urea production reflected decreased production of ureogenic substrates by peripheral tissues, we might postulate that the increased amino acid supply generated via augmented protein breakdown found in our study is reused locally for protein synthesis.

All of our subjects underwent a therapeutic trial with rhGH at a dose of 75 μg/kg given three times a week. There are limited systematic data available on growth hormone secretory rates in childhood. Based on the recently published data of Albertsson-Wikland *et al.* (63), the growth hormone treatment doses used in our study were within the normal secretory range for children of the same height and pubertal status. Based on similar data recently reported by Rosenbaum and Gertner (64), the treatment dose used in our study may have been as high as three times the child's usual secretory rate.

The response to therapy was highly variable with the change in height Z score ranging from 0 to +1.6 after 1 y of treatment with rhGH. The two children with the greatest increase in nitrogen retention had the greatest increment in height velocity Z score at 1 y (Fig. 6). All seven children whose height velocity Z scores increased by more than 6 had a greater than 25% decline in ^{15}N excretion. The two children with decrements in ^{15}N excretion less than 10% increased their growth rate Z score by less than 4. On the other hand, most children showed a greater than 25% decrease in ^{15}N excretion but increased their height velocity Z scores by less than 4 (Fig. 6). Thus, overall, the weak but significant correlation demonstrated between ^{15}N retention and height velocity Z score is not a useful predictor of height velocity response for individual subjects.

There may be several reasons for the lack of demonstrated correlation. First, the children received a fixed dose of growth hormone for the acute challenge. Based on the recently reported growth hormone secretion rates mentioned above (63, 64), this dose may have represented a variable stimulus to nitrogen retention depending on the magnitude of the child's own prior endogenous secretory rate. Similarly, growth hormone may have induced a variable degree of insulin resistance, both with regard to its effects on amino acid metabolism and in reference to its role in carbohydrate homeostasis. The almost immediate increase seen in urinary C-peptide excretion supports the rapid development of some decline in tissue sensitivity to insulin. On the other hand, it is likely that more long-term adaptive and accommodative changes in fuel metabolism induced by chronic growth hormone treatment superceded any temporary acute effects of growth hormone on nitrogen kinetics. In addition to alterations in insulin sensitivity mentioned above, these include alterations in energy intake and/or expenditure, as well as peripheral adipose tissue lipolysis. Only serial studies of the kinetics of amino acid, glucose, and lipid fuels before and during growth hormone supplementation can help resolve these issues.

In conclusion, 37 short children exhibited dramatic but highly variable changes in body protein kinetics in response to a short course of growth hormone that increased whole body protein turnover, synthesis, and breakdown. The growth rate increments in response to long-term growth hormone treatment were also variable and were not predictable from the acute nitrogen kinetic changes induced by growth hormone administration. Presumably, this variability is due to physiologic mechanisms that regulate body nitrogen economy during growth. Understanding the mechanisms responsible for the variability will provide insight into the relationships between metabolic fuel transport, energy balance, and protein dynamics and their regulation by growth hormone in growing children.

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