Acute effects of growth hormone administration: vitamin A and visceral protein concentrations

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Abstract. We determined albumin, total protein, somatomedin C, retinol binding protein (RBP), transferrin, prealbumin, and complement (C₃) concentrations in serum, and vitamin A concentrations in plasma before initiation of growth hormone (GH) therapy and 13 h after each of 4 daily injections of GH (0.1 U/kg) in 6 GH deficient children. Levels of total protein, albumin, and transferrin did not change during the first week of GH administration, however, transferrin levels rose after 6 weeks of GH therapy. Initial somatomedin C levels were below the normal range (0.19 ± 0.03 U/ml), and rose with GH administration in 4 of the 6 subjects, resulting in a post-treatment mean of 0.56 ± 0.23 U/ml. With GH administration vitamin A and RBP concentrations decreased from initial values (P < 0.05), and reached a plateau after 2 injections of GH, while there was no change in concentrations of other proteins. There was a significant linear correlation (P < 0.05) between the levels of vitamin A and RBP before and after GH administration. We conclude that GH administration results in a selective and marked reduction in the concentrations of plasma vitamin A and serum RBP.

A number of visceral proteins have been shown to be sensitive to nutritional state. These include albumin, transferrin, fibrinogen, retinol binding protein, and prealbumin, as well as acute phase glycoproteins and ceruloplasmin. It has been well demonstrated that administration of growth hormone (GH) to GH deficient children results in an increase in plasma concentrations of somatomedin (Copeland et al. 1980; Kemp et al. 1981; Dean et al. 1982), a nutritionally dependent visceral protein (Hintz et al. 1978). However, the increase in somatomedin levels has not been shown to correlate with growth (Rosenfeld et al. 1981). Transferrin has growth promoting activity in cell culture studies (Hayashi & Sato 1976), and may play a role in growth hormone-dependent growth (Donnadieu et al. 1980). Vitamin A is essential for normal growth, but the relationship between vitamin A and GH has not been well studied. We have examined the effect of GH administration on levels of vitamin A and specific visceral proteins in 6 GH deficient children.

Materials and Methods

Study subjects

Six GH deficient boys, ranging in age from 3 8/12 to 16 0/12 were studied. Although 3 of the subjects received prior GH therapy (0.1 U/kg, 3 times per week), none of the subjects had received GH for 3 months before the beginning of the study. The remaining 3 subjects had received no previous growth hormone therapy. All subjects had been shown to be unable to raise serum GH concentrations to 7 ng/ml in response to L-DOPA, infusion of arginine, and bolus injection of insulin. Two subjects had additional pituitary deficiencies; both received desmopressin (DDAVP®, Armour) for diabetes insipidus, and one required additional replacement of l-thyroxine and hydrocortisone. Four of the patients were diagnosed as having idiopathic GH deficiency; of the remaining 2 subjects, one was hypopituitary secondary to a craniopharyngioma, and the other secondary to a pilocytic astrocytoma. Subjects were evaluated at 3 month intervals, and heights were measured on a stadiometer. The annual growth rate was calculated from the height increment observed at the 6 month visit.

Informed consent was obtained from the parents of all

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subjects. These investigations were approved by the University of South Alabama Committee for the Protection of Human Subjects.

Experimental protocol
Blood samples for determination of vitamin A and specific visceral protein concentrations were collected from each of the 6 GH deficient subjects 2 weeks prior to initiation of GH therapy. Immediately following collection of blood samples the subjects were placed on a diet which supplied 1.5 times the calculated requirement for both total calories and protein. They remained on this diet for the duration of this study and kept a daily food diary. A blood sample was collected at 09.00 h on day 1 prior to administration of GH. hGH (0.1 U/kg) was administered im at 20.00 h on days 1–4, followed by continued administration at a dose of 0.1 U/kg, 3 times per week. Venous specimens were collected at 09.00 h on days 1–5. An additional specimen was collected after 6 weeks. The blood was promptly centrifuged, the plasma separated, and stored at −70°C in the dark until assay.

Determination of vitamin A levels and serum protein concentrations
Vitamin A was determined fluorometrically after separation on silicic acid as described by Garry et al. (1970). Total protein was determined colorimetrically using a Coomassie Brilliant Blue G-250 method (Bradford 1976) with reagents purchased from Bio-Rad (Richmond, CA). Albumin was measured by the Brom cresyl Green method (Doumas & Biggs 1972). Retinol binding protein, transferrin, complement (C₃), and prealbumin concentrations were quantitatively estimated by the single radial immunodiffusion technique of Mancini et al. (1965), with prepared plates obtained from Calbiochem-Behring Corporation (La Jolla, CA). Serum somatomedin-C (SM-C) levels were measured by radioimmunoassay; these assays were carried out by Endocrine Sciences (Tarzana, CA).

Statistical methods
Multiple mean comparisons of paired data were analyzed using randomized block analysis of variance, the Dunnett’s procedure. Unpaired comparisons of means from the two groups were made using unpaired t-test. Level of significance was chosen as *P* < 0.05.

Results

Effect of GH therapy on total protein, albumin, complement (C₃), and transferrin
Serum concentrations of total protein and albumin are shown in Fig. 1, and serum concentrations of transferrin are shown in Fig. 2. Two weeks prior to receiving GH the subjects were placed on a diet

![Figure 1](https://via.placeholder.com/150)

Fig. 1.
Response of total protein, serum albumin, and complement (C₃) to GH administration. hGH was administered for 4 consecutive days at a dose of 0.1 U/kg, followed by 0.1 U/kg 3 times per week, as described in Materials and Methods. Samples were drawn 13 h after each administration of GH. ● serum total protein; ○ complement (C₃); ▲ serum albumin. Each value represents the mean ± SEM.
which provided 1½ times their calculated requirements for calories and protein. This did not significantly change the serum concentrations of total protein, albumin, or transferrin. Further, four injections of GH did not change concentrations of these proteins from basal pre-GH values. Six weeks of GH therapy did not result in changes in total protein, albumin, complement (C3), or prealbumin levels; however, transferrin increased to a level significantly higher than pre-treatment concentrations ($P < 0.01$).

**Effect of GH therapy on prealbumin, retinol binding protein, and vitamin A**

Serum concentrations of prealbumin are shown in Fig. 2, and serum concentrations of retinol binding protein and plasma concentrations of vitamin A are shown in Figs. 3 and 4. Increased caloric and protein intake for 2 weeks prior to the beginning of growth GH therapy did not result in a change in concentrations of prealbumin, retinol binding protein or vitamin A. Therapy with GH did not result in a significant change in prealbumin concentrations. The initial injection of GH resulted in a decrease in concentrations of both retinol binding protein and vitamin A. This did not result in a change in the serum concentrations of total protein, albumin, or transferrin. Further, four injections of GH did not change concentrations of these proteins from basal pre-GH values.

**Fig. 2.**
Response of serum prealbumin and serum transferrin to GH administration. hGH was administered for 4 consecutive days at a dose of 0.1 U/kg, followed by 0.1 U/kg 3 times per week, as described in Materials and Methods. Samples were drawn 13 h after each administration of GH. • serum prealbumin; ▲ serum transferrin; Each value represents the mean ± SEM. * $P < 0.05$.

**Fig. 3.**
Response of serum retinol binding protein to GH administration. hGH was administered for 4 consecutive days at a dose of 0.1 U/kg, followed by 0.1 U/kg 3 times per week, as described in Materials and Methods. Samples were drawn 13 h after each administration of GH. Each value represents the mean ± SEM. * $P < 0.05$. 
Response of serum vitamin A to GH administration. hGH was administered for 4 consecutive days at a dose of 0.1 U/kg, followed by 0.1 U/kg 3 times per week, as described in Materials and Methods. Samples were drawn 13 h after each administration of GH. Each value represents the mean ± SEM. *P < 0.05. ‡P < 0.01.

protein and vitamin A. The level of retinol binding protein fell further after the second injection, and remained at the lower level throughout the duration of the study. Vitamin A levels fell after the first and second GH injections and remained low throughout the first week of therapy. Unlike the response of retinol binding protein to GH administration, the vitamin A levels rose with subsequent GH administration, so that the concentration of vitamin A after 6 weeks of therapy was indistinguishable from the concentration at the beginning of the study. As shown in Fig. 5 there was a strong correlation between the plasma vitamin A levels and serum concentrations of retinol binding protein (r = 0.79).

Response of growth and SM-C to GH therapy in GH deficient patients
Levels of SM-C were determined before and after four daily injections of GH in the 6 GH deficient subjects. In addition growth rates were determined prior to therapy and after receiving 6 months of GH replacement. These results are shown in Table 1. Initial levels of SM-C were all in the hypopituitary range. After four injections of GH, 4 of the 6 patients demonstrated a significant rise in SM-C concentrations, while SM-C levels in the other 2 patients remained constant. The 2 patients who did not respond to GH administration with an increase in SM-C concentrations were the 2 patients who were hypopituitary as a result of intracranial tumours. All 6 subjects responded to GH therapy with an increase in growth rate. There was no significant correlation between the rise in SM-C concentrations and growth rate, or the rise in SM-C concentrations and changes in either RBP or vitamin A concentrations.

Discussion
We have studied the effect of GH administration on growth rate, visceral protein concentrations, and vitamin A concentrations in 6 GH deficient subjects. All 6 subjects responded to GH therapy
with a marked increase in growth rate. Four of the 6 subjects responded acutely with an increase in serum SM-C levels. There was no correlation between the change in serum SM-C levels and growth rate. This is consistent with the reports of Rosenfeld et al. (1981) and Plotnick et al. (1983) that acute somatomedin response to GH therapy does not correlate with growth rate.

Concentrations of serum total protein, albumin, complement (C₃), and transferrin in GH deficient patients did not change during the first week of GH administration. However, 6 weeks after initiating GH therapy, serum transferrin concentrations were significantly higher than the pre-treatment basal values, suggesting that transferrin is subject to control by GH. This observation would indicate that the effect of GH on concentrations of transferrin takes place in a period of more than one week rather than hours, as has been suggested by Donnadieu et al. (1980).

GH therapy did have an effect on levels of vitamin A and retinol binding protein. Ordinarily, vitamin A is absorbed from the gut and transported together with chylomicrons to the liver. From there it is released into the serum bound to retinol binding protein and the thyroxine-binding prealbumin (Rask et al. 1980). Excretion of retinol is primarily into bile, while degradation of retinol binding protein takes place in the kidney, and appears to be dependent upon concentrations of prealbumin (Vahlquist 1972). With GH therapy we have observed a decrease in concentrations of vitamin A and retinol binding protein, while levels of prealbumin did not change from pre-treatment values. In addition, there was a strong correlation between concentrations of vitamin A and retinol binding protein, while there was no significant linear correlation between vitamin A and prealbumin. This observation is consistent with a report (Smith F R et al. 1973) which has suggested that retinol binding protein concentration may be a reliable indicator of vitamin A level.

The effect of GH on plasma levels of retinol binding protein could result from three possible mechanisms. First, therapy with GH could directly cause a decrease in hepatic synthesis of retinol binding protein, perhaps as a result of the GH mediated increase in somatomedin synthesis diverting substrates away from synthesis of retinol binding protein. We observed no correlation between the fall in either retinol binding protein or vitamin A concentrations and the rise in somatomedin levels, which suggests that the effect of GH on retinol binding protein and vitamin A is not mediated by somatomedin. Although we observed a decrease in the level of RBP, we did not observe a decrease in the levels of any other visceral protein. Of all the proteins examined retinol binding protein has the shortest half-life (0.5 days) and the smallest pool size (5 mg/kg) (Ingenbleek et al. 1975; Schultz & Heremans 1966). It appears that a new steady-state concentration of retinol binding protein is achieved after 2 days (about 4 half-lives). If GH had a general effect of decreasing synthesis of visceral proteins other than somatomedin, we would at least expect to see a fall in the level of prealbumin, which has a half-life of 1.9 days (Schultz & Heremans 1966). We observed prealbumin concentrations for more 2 half-lives without noting a change. Further, complement (C₃) has a half-life similar to prealbumin (1.4 days), and we did not observe an effect of GH therapy on concentrations of this protein. Therefore, it does not appear that we can account for the fall in the level of RBP by a general decrease in hepatic synthesis of visceral proteins.

A second possible effect of GH would be to increase the degradation of retinol binding protein. The half-life of free retinol binding protein is about 3.5 h, while the half-life of retinol binding protein bound to prealbumin is about 12 h (Vahlquist 1972). However, an increase in the degrada¬

Table 1.
Effect of GH therapy on growth rate and serum somatomedin-C levels.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before GH</th>
<th>After GH</th>
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<tbody>
<tr>
<td></td>
<td>Growth rate (cm/year)</td>
<td>SM-C (U/ml)</td>
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<tr>
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<td>3.3</td>
<td>0.32</td>
</tr>
<tr>
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<td>2.3</td>
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</tr>
<tr>
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<td>0.12</td>
</tr>
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<td>1.7</td>
<td>0.17</td>
</tr>
<tr>
<td>6</td>
<td>0.0</td>
<td>0.24</td>
</tr>
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Mean ± SEM 0.19 ± 0.03 0.56 ± 0.23

Serum SM-C by radioimmunoassay.
tion of RBP would require a decrease in the pool of prealbumin, which we did not observe.

A third possible explanation is that GH could primarily cause a decrease in the level of vitamin A with a resultant decrease in retinol binding protein concentration. In vitamin A deficiency vitamin A is necessary for the further processing of retinol binding protein molecules; in this state concentrations of retinol binding protein increase in the liver while decreasing in the serum. Vitamin A therapy results in reversal of this process by permitting mobilization of stored retinol binding protein (Smith J E et al. 1973; Peterson et al. 1973). Therefore, if GH results in a decrease in levels of vitamin A, there would be an increase in storage of vitamin A in the liver with a decrease in serum retinol binding protein levels. While these data do not rule out other mechanisms, our finding that levels of vitamin A and retinol binding protein decrease in response to GH administration without a change in prealbumin levels would be consistent with a mechanism in which vitamin A levels first decrease, followed by a fall in levels of retinol binding protein.

The physiological implications of the decrease in vitamin A and retinol binding protein in response to GH administration remain unclear. Normal levels of vitamin A appear to be necessary for normal growth; however, retinoic acid has been shown to have inhibitory as well as stimulatory effects (Lotan 1980). Thus, it is possible that a decrease in the level of vitamin A could enhance growth in response to GH. Our observations demonstrate a relationship between GH and vitamin A, suggesting an important role for vitamin A in GH dependent growth.

Acknowledgments

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