Growth hormone increases serum 1,25-dihydroxyvitamin D levels and decreases 24,25-dihydroxyvitamin D levels in children with growth hormone deficiency

Shi Wei, Hiroyuki Tanaka, Toshihide Kubo, Taeko Ono, Susumu Kanzaki and Yoshiki Seino

Department of Pediatrics, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, Japan

(Correspondence should be addressed to Y Seino, Department of Pediatrics, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, Japan)

Abstract

The influence of growth hormone (GH) on calcium-phosphorus metabolism and modulation of vitamin D metabolism has been demonstrated, but the mechanism remains unclear. We investigated the effect of a 6-month course of GH therapy on vitamin D and mineral metabolism in twelve GH-deficient children. Before GH therapy, levels of vitamin D metabolites and other biochemistry data were within normal ranges. All patients responded to GH therapy with increased growth velocity. 1,25-Dihydroxyvitamin D levels increased after 1 month of treatment and remained at these higher levels, with a significant increase found at 3 months (P < 0.05), whereas 24,25-dihydroxyvitamin D levels were decreased at 1 and 3 months, the latter being a significant decrease (P < 0.05), and then returned to the baseline levels at 6 months. 25-Hydroxyvitamin D levels did not change significantly. A significant increase in serum insulin-like growth factor-I (IGF-I) levels occurred during the 6 months of treatment (1 month, P < 0.01; 3 and 6 months, P < 0.001). Serum parathyroid hormone (PTH) levels decreased significantly at 3 and 6 months (3 months, P < 0.01: 6 months, P < 0.05). Serum calcium and phosphorus levels did not change significantly. Significant increases were found in the urinary calcium/urinary creatinine ratio (3 and 6 months, P < 0.05) and the percent tubular reabsorption of phosphorus levels (1 and 3 months, P < 0.05). The results of this study confirmed the actions of GH on renal tubules with increases in calcium excretion and phosphorus reabsorption, and indicate that the action of GH on modulating vitamin D metabolism may be IGF-I mediated, not PTH mediated.

European Journal of Endocrinology 136 45–51

Introduction

In early studies, growth hormone (GH) was demonstrated to act on mineral metabolism with an increase in intestinal calcium absorption and urinary calcium excretion, and a decrease in urinary phosphorus excretion (1–6). The partial vitamin D-like actions of GH have led us to suggest that GH exerts its effect by modulating vitamin D metabolism. A number of observations seemed to support this hypothesis. Low serum levels of 1,25-dihydroxyvitamin D (1,25-(OH)2D) have been reported in hypophysectomized rats, and GH has been shown to increase serum 1,25-(OH)2D in these rats (7, 8). Another study demonstrated that in hypophysectomized rats, in which renal conversion of 25-hydroxyvitamin D (25OHD) to 1,25-(OH)2D was markedly reduced and conversion of 25OHD to 24,25-dihydroxyvitamin D (24,25-(OH)2D) was markedly increased, treatment with rat GH resulted in a significant increase in renal conversion of 25OHD to 1,25-(OH)2D and a significant decrease in conversion of 25OHD to 24,25-(OH)2D (9). Administration of porcine GH to pigs increased serum levels of 1,25-(OH)2D (10, 11) and decreased those of 24,25-(OH)2D (10).

Similar results have been observed in human studies on 1,25-(OH)2D, the hormonal form of vitamin D. Patients with acromegaly show elevated serum levels of 1,25-(OH)2D, which decrease with the inhibition of GH secretion (12). Several studies have shown the acute effects of GH administration on the increase in serum 1,25-(OH)2D in GH-deficient children (13), normal adults (5) and elderly people (4). It has been well established that a late and/or continuous elevation of serum 1,25-(OH)2D levels accompanies long-term GH administration in subjects with or without GH deficiency (6, 14, 15). However, there are few reports of human studies on other vitamin D metabolites associated with GH treatment and results of animal studies have been inconsistent. In acromegaly, elevated 24,25-(OH)2D levels fell with the inhibition of GH secretion, but two other studies found no change in serum 24,25-(OH)2D levels during GH treatment (2, 3). The present investigation was designed to define the long-term effects of GH administration on vitamin D and mineral metabolism.
Subjects and methods

Subjects

Twelve children (aged 7.5–13.8 years, 10 boys and 2 girls) with isolated GH deficiency were studied. All subjects were of normal weight and height and had no trauma at birth. All subjects had normal renal and liver function and had normal daily lives. None had a history of malignancy or took any medication known to interfere with vitamin D or mineral metabolism. Each had GH peaks < 10 ng/ml in response to at least two provocative pharmacologic stimuli. In addition, four subjects had no or markedly reduced responses to clonidine and glucagon tolerance tests, three to insulin and clonidine tests, three to levodopa and arginine tests, one to insulin and glucagon tests and one to insulin and levodopa tests. All subjects remained prepubertal throughout the study period. Informed consent for the study was obtained from the parents of each child.

Study protocol

Blood was obtained at 0900 h for determination of serum calcium, phosphorus, creatinine, parathyroid hormone (PTH), insulin-like growth factor-I (IGF-I), and vitamin D metabolites. Urine was collected from 0700–0900 h for measurements of calcium, phosphorus and creatinine. Recombinant human GH (0.5 IU/kg per week) was injected subcutaneously in six fractions per week. Blood and urine samples were obtained for vitamin D metabolites and the biochemical measurements before and after 1, 3 and 6 months of GH therapy. We also evaluated the urinary calcium/urinary creatinine ratio (UCa/Ucr) and the percent tubular reabsorption of phosphorus (TRP%) which was derived by the following equation:

$$\text{TRP}_i\% = \left(1 - \frac{\text{UPI}}{\text{PPI}} \times \frac{\text{PCR}}{\text{UCr}}\right) \times 100\%$$

where $P$ = plasma and $i$ = inorganic.

Measurements

Quantification of 25OH D, 1,25-(OH)2 D and 24,25-(OH)2 D was carried out as previously reported (16). In brief, vitamin D metabolites were purified with C-18/OH cartridges (Analytichem Int., Harbor City, CA, USA) and Sep-Pak silica cartridges (Waters Corporation, Milford, MA, USA), and were quantitated by radioreceptor assay using bovine mammary gland receptor for 1,25-(OH)2 D and competitive protein binding assay for 25OH D and 24,25-(OH)2 D. A highly sensitive radiolmmunoassay for mid-region and carboxyl terminal of PTH (M-PTH) was performed using a PTH kit (Yamasa) YSI-7707 (Yamasa Shoyu Company, Chiba, Japan). Serum IGF-I levels were determined by radioimmunoassay (Ciba

Table 1 The baseline biochemical data of patients participating in the study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td>Corrected calcium (mg/dl)</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>203.3 ± 68.9</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>237.0 ± 127.2</td>
</tr>
<tr>
<td>25OH D (ng/ml)</td>
<td>26.3 ± 10.9</td>
</tr>
<tr>
<td>1,25-(OH)2 D (pg/ml)</td>
<td>59.4 ± 10.2</td>
</tr>
<tr>
<td>24,25-(OH)2 D (ng/ml)</td>
<td>3.9 ± 2.9</td>
</tr>
<tr>
<td>UCa/Ucr ratio (mg/mg)</td>
<td>0.14 ± 0.07</td>
</tr>
<tr>
<td>TRP%</td>
<td>92.1 ± 2.9</td>
</tr>
</tbody>
</table>

Figure 1 Changes in serum 1,25-(OH)2 D, 24,25-(OH)2 D and 25OH D during GH treatment. *$P < 0.05$ vs pretreatment (pre).
Corning Diagnostica, Tokyo, Japan). Serum calcium and phosphorus and urinary calcium and phosphorus were determined by an automatic analyzer. Corrected calcium levels were used to express serum ionized calcium levels, evaluated by serum calcium minus albumin levels, and serum albumin levels were assayed using an albumin B-test Wako kit (Wako Pure Chemical Industries Ltd, Osaka, Japan).

**Statistical analysis**

The results of pretreatment and intra-treatment examinations were analyzed for significance by two-tailed paired t-test, and \( P < 0.05 \) was considered significant. All results are given as means ± standard deviation.

**Results**

The biochemistry data at baseline are given in Table 1. Before GH therapy, serum levels of vitamin D metabolites, other biochemistry data and TRP% levels were within the normal range in all patients. All patients responded to GH therapy with increased growth velocity (4.7 ± 0-9 cm/year before and 7.4 ± 1.8 cm/year at 6 months, \( P < 0.001 \)).

The changes in serum levels of 1,25-(OH)\(_2\)D, 24,25-(OH)\(_2\)D and 250HD during GH therapy are shown in Fig. 1. 1,25-(OH)\(_2\)D levels began to increase after 1 month of treatment and remained high up to 6 months, with a significant increase found at 3 months (670 ± 130 pg/ml, \( P < 0.05 \) vs pretreatment). 24,25-(OH)\(_2\)D levels decreased significantly at 3 months (2-1 ± 1.2 ng/ml, \( P < 0.05 \)), and then returned to baseline levels at 6 months. No significant variations in 25OHDF levels were found during GH treatment. A good correlation was found between the ratio of the increase in 250HD and 24,25-(OH)\(_2\)D at 6 months (Fig. 2).

During GH treatment, no significant variations were found in serum levels of calcium, corrected calcium or phosphorus levels (Fig. 3). Serum PTH levels were decreased at 1 month and remained at low levels until 6 months, with significantly lower levels observed at 3 months (147.5 ± 41.6, \( P < 0.01 \)) and 6 months (162.0 ± 44.7, \( P < 0.05 \)) (Fig. 4). Significant increases in serum IGF-I levels occurred during the 6 months of treatment (1 month: 339.9 ± 143.2, \( P < 0.01 \); 3 months: 377.0 ± 179.9, \( P < 0.001 \); 6 months: 328.3 ± 177.9, \( P < 0.001 \)) (Fig. 5).

The UCa/UCr ratio rose slowly after the start of treatment, and significantly higher levels were found at 3 and 6 months (0.27 ± 0.16 and 0.23 ± 0.09, \( P < 0.05 \)). Concomitantly, TRP% levels increased significantly at 1 and 3 months of treatment (94.6 ± 4.0%...
and 94.9 ± 2.0%, P < 0.05) and fell at 6 months even though they remained higher than before treatment (Fig. 6).

**Discussion**

Previous studies have reported that high dose GH therapy raised 1,25-(OH)₂D levels acutely in GH-deficient children (13), normal adults (5) and elderly people (4). Unfortunately, we did not measure the serum levels of vitamin D metabolites until the end of the first month of GH treatment. Late and/or continuous increases of serum 1,25-(OH)₂D levels have also been observed during long-term GH administration in short children without GH deficiency, and in GH-deficient adults and children where 1,25-(OH)₂D levels were reported to be increased after 8 weeks (14), 6 months (15) and 12 months of treatment (6). The present study partially confirmed these findings, showing that 1,25-(OH)₂D levels were increased after 1 month of treatment, and were significantly increased at 3 months. However, a study of the 3-month course of
GH therapy in GH-deficient children, at a lower dose than our study and others, found no change in serum 1,25-(OH)₂D levels (3). In contrast, Chipman et al. (2) noted a decrease in 1,25-(OH)₂D levels after 5–14 months of GH treatment in GH-deficient children. However, serum levels of 1,25-(OH)₂D in most subjects varied only within a narrow range in their study. Thus, the lower sensitivity and higher inter assay variations of their assay may have contributed to the variation in serum 1,25-(OH)₂D levels.

The mechanism involved in the increased circulating levels of 1,25-(OH)₂D remains unclear. Since the primary physiological regulators of 1,25-(OH)₂D production are serum calcium, inorganic phosphorus and PTH (17, 18), the stimulation of renal 1α-hydroxylase activity may, theoretically, be considered as follows. First, GH or IGF-I might act on the kidneys to increase serum phosphorus levels by increasing urinary phosphorus reabsorption, and this may cause an increase in the serum PTH level which stimulates 1α-hydroxylase activity. However, PTH levels did not rise in our study, suggesting that it is not a PTH-mediated effect. Secondly, GH or IGF-I might directly regulate mineral metabolism through the increased rate of bone growth, resulting in a decrease in serum phosphorus which is a stimulator of 1α-hydroxylase activity; however, there were no changes in serum calcium or phosphorus levels in our study. Thus, it is possible that GH or IGF-I stimulates renal 1α-hydroxylase activity directly. Recently, a number of animal studies have suggested that the GH-dependent increase in serum 1,25-(OH)₂D under conditions of dietary phosphorus deprivation is mediated by IGF-I (19, 20), and similar results have been obtained in GH-deficient patients (21). Data obtained in phosphorus-replete hypophysectomized rats also indicated the possible role of IGF-I in mediating the effect of GH on the renal handling of phosphorus and the production of 1,25-(OH)₂D (22). The increased levels of 1,25-(OH)₂D induced by pharmacological doses of GH are associated with the elevation of IGF-I levels in our study and other clinical studies without restriction of dietary phosphorus (4, 5, 14). Portale et al. (23–25) demonstrated that dietary phosphorus can regulate the renal production and serum level of 1,25-(OH)₂D, and that it is an important determinant of the serum level of phosphorus, providing evidence that the regulation is mediated by modulation of the serum level of phosphorus. Serum levels of phosphorus did not increase significantly in our study, suggesting that this is the effect of non-restriction of dietary phosphorus.

The cause of the decreased synthesis of 24,25-(OH)₂D also remains unclear. 1,25-(OH)₂D has been shown to induce renal 25OHD-24-hydroxylase, and PTH has been reported to decrease renal 24-hydroxylase activity (26–28), indicating a biphasic response of 24,25-(OH)₂D. We suggest that this is due to the inhibitory effect of GH or IGF-I on renal 24-hydroxylase. However, the changes in 24,25-(OH)₂D levels in the late phase (after 3 months) seemed to be due to the changes in 25OHD levels, since there was a good correlation between the ratio of the increase in 25OHD and that of 24,25-(OH)₂D levels at 6 months.

We found a decrease in serum PTH after GH treatment, coinciding with the increase in serum 1,25(OH)₂D. van der Veen and Netelenbos (29) also reported a decrease in the PTH level during GH administration in adults. However, Saggese et al. (6) noted that intact PTH levels increased continuously during long-term GH administration in GH-deficient patients. Previous studies have demonstrated that intact PTH secretion is pulsatile (30), responds to rapid changes of ionized calcium and inorganic phosphorus levels (30, 31), and is correlated to GH levels (32). Thus, it is possible that intact PTH, which was measured in the morning, showed acute variation due to GH infused during the night. However, M-PTH which has a longer half-life has been shown to be a better indicator of the long-term effects on the variation of PTH than intact.

Figure 6 Effects of GH treatment on urinary Ca/Cr levels and TRP%. *P < 0.05 vs pre.
PTH (33). Thus, it may be necessary to measure M-PTH levels to clarify the biological effects of long-term drug administration on the dynamic secretion of PTH.

The actions of GH on urinary calcium and phosphorus excretion may be a direct effect rather than a PTH-independent mechanism, since PTH should exert contrary effects on urinary calcium and phosphorus, and the effects on urinary phosphorus appeared earlier than changes in serum 1,25-(OH)₂D levels. The increase of TRPi% may play a role in providing high extracellular phosphorus levels for mineralization of bone matrix during GH administration.

In conclusion, our study confirms that GH acts on renal tubules resulting in an increase in urinary calcium excretion and urinary phosphorus reabsorption, which may be direct effects. We demonstrated that long-term pharmacological doses of GH increased the serum level of 1,25-(OH)₂D and decreased the serum level of 24,25-(OH)₂D. The exact mechanism by which GH affects renal hydroxylation and how it acts remains unclear, but we suggest that it is not a PTH-mediated effect.

Acknowledgements

We wish to acknowledge the expert assistance of Rumi Abe-Nojima and Yuko Okamoto. This work was supported in part by grants from the Ministry of Health and Welfare and the Ministry of Education of Japan.

References

19 Gray RW. Evidence that somatomedins mediate the effect of hypophosphatemia to increase serum 1,25-dihydroxyvitamin D₃ levels in rats. Endocrinology 1987 121 504–512.


Received 19 March 1996

Accepted 29 August 1996