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Different effects of physiologically and pharmacologically increased growth hormone levels on cholecalciferol metabolism at prepubertal age☆

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Abstract

The aim of the study was to investigate the influence of physiologically and pharmacologically increased plasma growth hormone (GH) levels on cholecalciferol metabolism at prepubertal age. Three groups of dogs raised on the same diet were studied from weaning till 21 weeks of age, i.e., small breed dogs ($n = 7$, control group); large breed dogs with 15-fold greater growth rates compared to the control group ($n = 8$, LB-group); and small breed dogs treated with pharmacological doses of growth hormone ($n = 6$, GH-group; 0.5 IU GH per kg body per day) from 12 to 21 weeks of age. Excess of GH had the expected anabolic effect on growth rate and phosphate sparing. Increased plasma GH levels in the LB- and GH-groups versus the control group were accompanied by (1) greater plasma insulin-like growth factor I (IGF-I) levels, (2) greater plasma 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) levels, and (3) lower plasma $24,25(OH)_2D_3$ levels. In the LB-group, excess of GH favored plasma $1,25(OH)_2D_3$ levels by decreasing the clearance of $1,25(OH)_2D_3$, whereas in the GH-group by increasing the production of $1,25(OH)_2D_3$. The lowered plasma $24,25(OH)_2D_3$ levels in the LB- and GH-groups were likely attributed to a competitive inhibition of the production of $24,25(OH)_2D_3$ by GH and/or IGF-I.

Keywords: Growth hormone; 1,25-Dihydroxycholecalciferol; 24,25-Dihydroxycholelcalciferol; Vitamin D_3 metabolism

1. Introduction

Cholecalciferol (vitamin D_3) is converted to 25-hydroxycholecalciferol ($25(OH)D_3$) in the liver and to $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ by a sequential hydroxylation primarily catalyzed by renal 1α-hydroxylase and 24-hydroxylase, respectively. These two hydroxylase enzymes are reciprocally regulated, with 1α-hydroxylase being directly responsive to a variety of regulators, including plasma levels of inorganic phosphate (P_i), growth hormone (GH), insulin-like growth factor I (IGF-I), and parathyroid hormone (PTH) [1–3]. Catabolism of $1,25(OH)_2D_3$ is mainly dependent upon 24-hydroxylase activity in the target organs of $1,25(OH)_2D_3$ and is regulated by $1,25(OH)_2D_3$ itself [4], P [5], $24,25(OH)_2D_3$ [6,7], PTH [8], and possibly by GH and IGF-I [9]. The general consensus is that pharmacological GH excess is accompanied by an increase in plasma $1,25(OH)_2D_3$ levels [10,11] due to stimulation of the renal production of $1,25(OH)_2D_3$ by IGF-I [2]. There is only circumstantial evidence on the down-regulating effect of GH excess on the plasma $24,25(OH)_2D_3$ levels [11,12].

The domestic dog, completely dependent on dietary intake of vitamin D_3 [13], represents a tremendous species-specific disparity in growth rate and hence in mature body weight (BW). Rapid growth rate and a large adult BW of 55–70 kg in the Great Dane has been associated with juvenile GH excess and high plasma IGF-I levels [14,15] compared to Miniature Poodles (adult BW of 7–8 kg). In order to evaluate the influence of physiological and pharmacological excess of GH on vitamin D_3 metabolism, small breed dogs were raised on a balanced diet and compared to large breed dogs with juvenile GH excess and to small breed dogs treated with GH. Growth regulating and calciotropic hormones were measured. The production and clearance rates of $1,25(OH)_2D_3$, as well as renal gene expression of 1α-hydroxylase and 24-hydroxylase, were determined.

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2. Materials and methods

2.1. Animals, diets and treatments

The Utrecht University Ethical Committee for Animal Care and Use approved all procedures. Fourteen Miniature Poodles and eight Great Danes (large breed, LB-group) were raised on an extruded diet formulated to meet the requirements for growing dogs [16]. Diets contained approximately 0.94 g Ca, 0.80 g P per 100 g diet on a dry matter basis, and 500 IU vitamin D_3 kg^{-1} diet. From 3 to 6 weeks of age, pups received their diet as a gruel in addition to the bitch milk and later on dry diet exclusively. Daily food intake was adapted biweekly to the actual BW provided at two times maintenance energy requirements of each dog [17]. Porcine GH (pGH), with an identical peptide structure as canine GH [18], was used in the study and was obtained from Dr. A.F. Parlow (National Hormone & Peptide Program, Torrance, CA). Starting at 12 weeks of age, GH was administrated in six Miniature Poodles (GH-group) subcutaneously (SC) once daily (at approximately 9:00 h) for 8 weeks as a sterile solution of 0.03 M sodium bicarbonate ($NaHCO_3$) in 0.15 M NaCl, adjusted to pH 9.5 at a dose of 0.5 IU GH per kg body per day. Likewise, eight Miniature Poodles (control group) received daily an equal amount of the vehicle of the solution.

2.2. Blood measurements

At 7, 13, 16, 19, and 21 weeks of age (prior to SC administration of GH), blood samples were collected after overnight food deprivation. Plasma total Ca and P levels were measured according to standard procedures (Beckman Industries Inc., Brea, USA). Basal plasma GH levels were defined as the median of six measure points, i.e., at 0 h, 30 min, 1, 2, 3, and 4 h, where 0 h is the time point of GH administration in the GH-group. GH was measured by a homologous RIA [19], with intra- and inter-assay CV of 3.8 and 7.2%, respectively. Total IGF-I levels were measured by a heterologous RIA [20], with intra- and inter-assay CV of 4.7 and 15.6%, respectively. $25(OH)D_3$ and $24,25(OH)_2D_3$ were quantitatively determined by a modified RIA (DiaSorin, Stillwater, Minnesota, USA) [21], with an intra- and inter-assay CV for $25(OH)D_3$ of 15.2 and 6.1%, respectively, and an intra- and inter-assay CV for $24,25(OH)_2D_3$ of 10.1 and 8.5%, respectively. $1,25(OH)_2D_3$ was quantitatively determined by a radioreceptor assay based on the method described by Reinhardt et al [22] and Hollis [23], with an intra- and inter-assay CV of 5.7 and 6.6%, respectively. PTH was measured using an immunoradiometric assay for intact PTH (iPTH; Nichols Institute, San Juan Capistrano, CA, USA) [24], with an intra- and inter-assay CV of 3.4 and 5.6%, respectively.

2.3. Endogenous metabolic clearance rate (MCR) and production rate (PR) of $1,25(OH)_2D_3$

At 19 weeks of age, the MCR of $1,25(OH)_2D_3$ was only determined in the control and LB-groups with the aid of 1α,25-dihydroxy[23,24(*n*)-^{3}H]cholecalciferol (^{3}H-$1,25(OH)_2D_3$; specific activity 10.5 GBq mg^{-1}; Amersham Pharmacia Biotech, UK) [21]. The PR of $1,25(OH)_2D_3$, expressed in pmol per kg BW per day, was derived from the formula:

$$PR = MCR \cdot \text{endogenous circulating } 1,25(OH)_2D_3$$

where endogenous circulating $1,25(OH)_2D_3$ is the plasma $1,25(OH)_2D_3$ level at 19 weeks of age.

2.4. Renal 1α-hydroxylase and 24-hydroxylase gene expression

At the end of the study, i.e., at 21 weeks of age, renal gene expression levels of 1α-hydroxylase and 24-hydroxylase were determined by real-time PCR and techniques described previously [21]. The amount of target (1α-hydroxylase and 24-hydroxylase) was divided by the amount of endogenous reference (β-actin) to obtain a normalized target value. Each of the normalized target values was divided by the normalized target value of the calibrator (control group) to generate *n*-fold relative expression levels.

2.5. Statistical analysis

Statistical analyses were performed using the SPSS for Windows 10.1 (SPSS Inc., Chicago, USA). Differences in growth curves between groups were analyzed with a covariance analysis. Differences between groups were tested in an ANOVA for repeated measurements (Tukey). Values were considered to be significant when $P < 0.05$. Results are presented as mean ± standard error of the mean (S.E.M.).

3. Results

Animals consumed the total daily amount of food provided during the entire study. BW-gain was significantly greater in the LB-group throughout the study and in the GH-group starting at 15 weeks of age versus the control group (Fig. 1).

3.1. Blood measurements

Plasma Ca levels did not differ between groups, whereas plasma P levels were significantly greater in the LB-group throughout the study and in the GH-group at 19 and 21 weeks of age versus the control group (Fig. 2). Basal plasma GH levels were significantly greater in the LB- and GH-groups for the duration of the study, whereas plasma IGF-I levels were greater in the LB-group for the duration of the

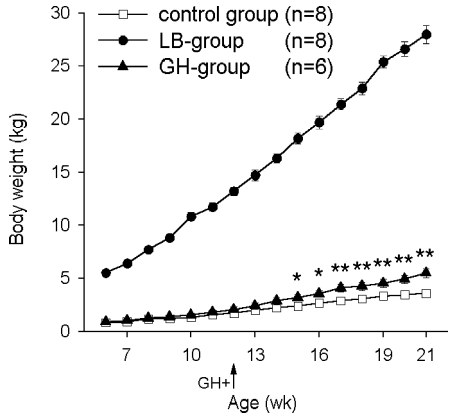

Fig. 1. Growth curve of a group of small breed dogs (control group), large breed dogs (LB-group), and of small breed dogs receiving 0.5 IU GH per kg body per day growth hormone (GH-group) starting in 12 weeks (arrow). Data are presented as mean ± S.E.M. All measured points in the LB-group were different with $P < 0.01$ vs. the control group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. the control group at the same age.

study and increased in the GH-group, starting at 16 weeks of age, versus the control group (Fig. 3). Before the initiation of GH administration to the GH-group plasma vitamin, D_3 metabolite levels did not differ between groups. Excess in plasma GH levels (LB- and GH-groups) was accompanied by lower plasma 25(OH)D_3 and 24,25$(OH)_2D_3$ levels and greater plasma 1,25$(OH)_2D_3$ levels versus the control group (Fig. 3). Plasma PTH levels did not differ between groups.

3.2. MCR and PR of 1,25$(OH)_2D_3$

At 19 weeks of age, MCR of 1,25$(OH)_2D_3$ was lower in the LB group versus the control group (0.27 ± 0.03 versus 0.65 ± 0.04 L per kg body per day, respectively, with $P < 0.01$), whereas PR of 1,25$(OH)_2D_3$ did not differ between these groups (66.9 ± 6.8 versus 79.4 ± 5.3 pmol kg BW^{-1} per day, respectively).

3.3. Gene expression of 1α-hydroxylase and 24-hydroxylase

At 21 weeks of age, renal 1α-hydroxylase gene expression was 12.9-fold ($P < 0.01$) greater in the GH-group versus the LB- and control groups, whereas renal 24-hydroxylase gene expression did not differ between groups.

4. Discussion

Physiological and pharmacological GH excess had the expected positive effect on BW-gain and sparing of P [25], and was accompanied by considerable differences in vitamin D_3 metabolism. Although all groups had the same vitamin D_3 intake per kg BW, plasma levels of 25(OH)D_3 were lower, of 1,25$(OH)_2D_3$ were greater, and of 24,25$(OH)_2D_3$ were lower in the LB- and GH-groups versus the control group.

Increased plasma GH levels of both endogenous and exogenous origin were accompanied by increased plasma IGF-I and 1,25$(OH)_2D_3$ levels compared to the control group according to the general consensus [10]; however, the underlying mechanism was surprisingly different. The increase in plasma 1,25$(OH)_2D_3$ levels in the GH-group due to increase in the renal production of 1,25$(OH)_2D_3$, as confirmed by the 12.9-fold increase in renal 1α-hydroxylase gene expression, is a well described phenomenon [25], with IGF-I as mediator [2], and is independent of PTH [26]. Accordingly, plasma PTH levels did not differ between groups. To the contrary, the increased plasma 1,25$(OH)_2D_3$ levels in the LB-group were not related to increased 1,25$(OH)_2D_3$ production as demonstrated by kinetic analysis and by

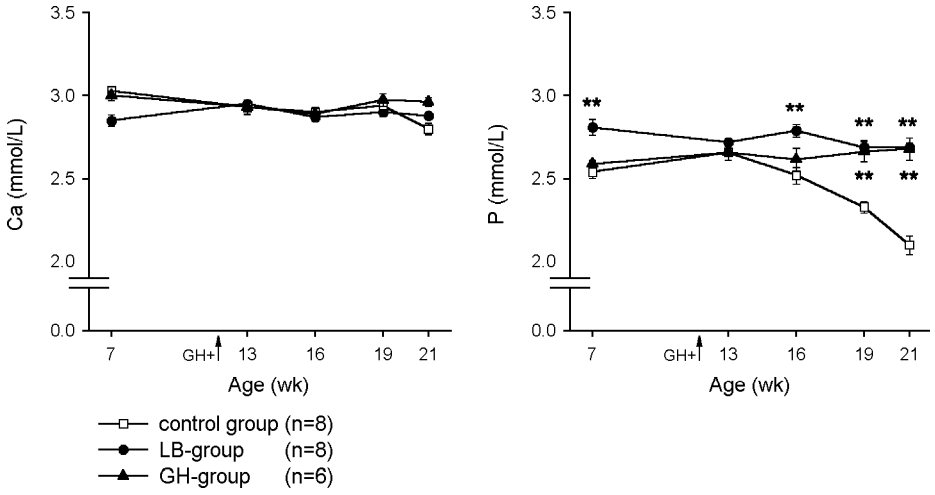

Fig. 2. Plasma levels of total calcium (Ca) and phosphate (P) in small breed dogs (control group), large breed dogs (LB-group), and in small breed dogs receiving 0.5 IU GH per kg body per day growth hormone (GH-group) starting in 12 weeks (arrow). Data are presented as mean ± S.E.M. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. the control group at the same age.

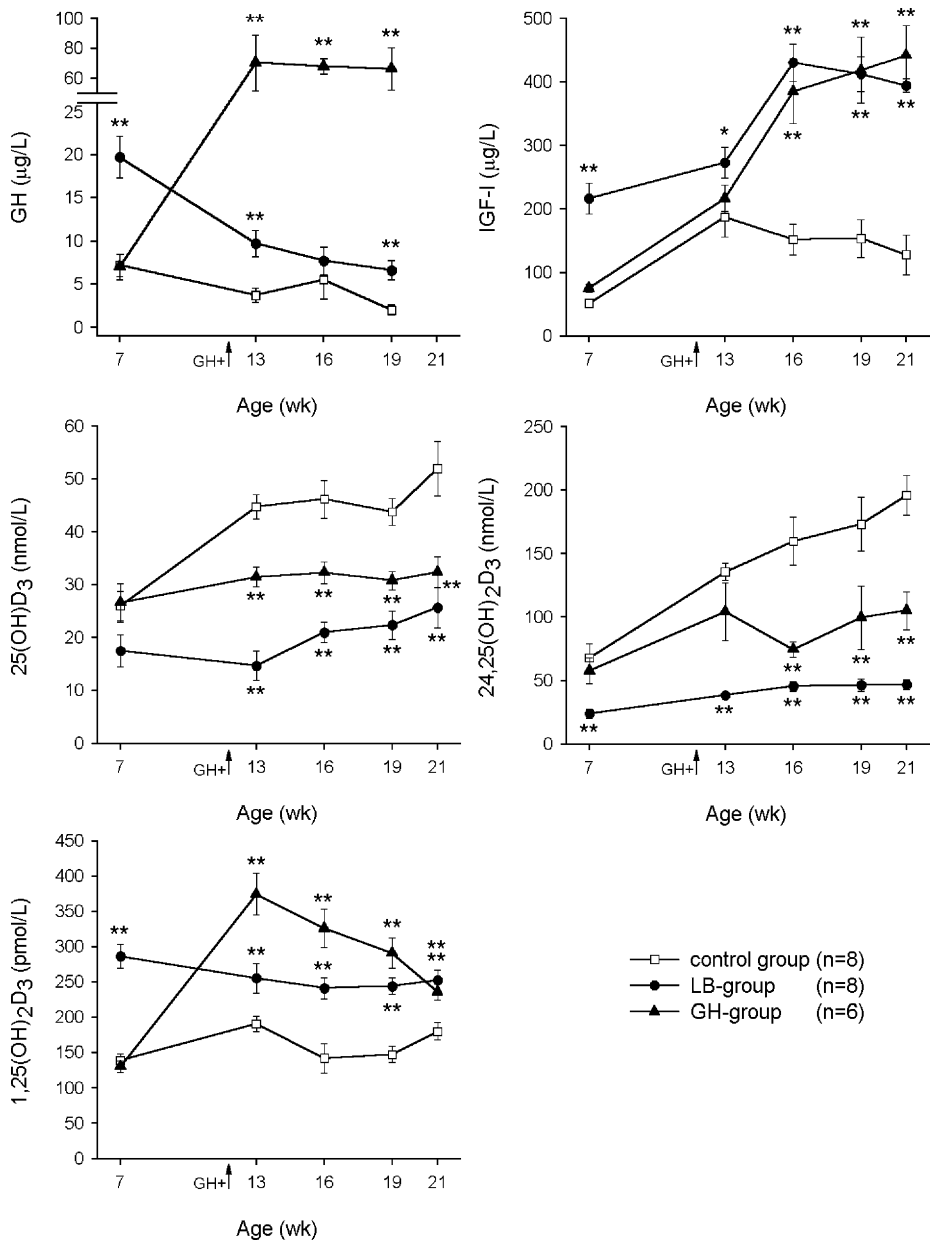

Fig. 3. Plasma levels of growth hormone (GH), insulin-like growth factor I (IGF-I), 25-hydroxycholecalciferol ($25(OH)D_3$), 24,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$), and $1,25(OH)_2D_3$ in small breed dogs (control group), large breed dogs (LB-group), and in small breed dogs receiving 0.5 IU GH per kg body per day growth hormone (GH-group) starting in 12 weeks (arrow). Data are presented as means ± S.E.M. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. the control group at the same age.

quantitative determination of the renal 1α-hydroxylase gene expression, but rather were related to a lower MCR of $1,25(OH)_2D_3$.

Catabolism of $1,25(OH)_2D_3$ is mainly dependent upon 24-hydroxylase activity in the target organs of $1,25(OH)_2D_3$. There is substantial circumstantial evidence suggesting a down-regulating effect of the GH–IGF-I axis on 24-hydroxylase influencing the production $24,25(OH)_2D_3$: hypophysectomy results in increased plasma $24,25(OH)_2D_3$ levels in rats [27], whereas GH treatment of hypophysectomized rats results in decreased plasma $24,25(OH)_2D_3$ levels, and IGF-I administration results in decreased renal 24-hydroxylase gene expression [9,28]. Accordingly, GH administration to GH-deficient children and to healthy pigs results in decreased plasma $24,25(OH)_2D_3$ levels [11,12]. The evidence can be further substantiated by the lower plasma $24,25(OH)_2D_3$ levels in the LB- and GH-groups compared to the control group, likely mediated by the increased levels of endogenous and exogenous GH, respectively.

Plasma $25(OH)D_3$ levels were lower in the LB- and GH-groups versus the control group. The production of

$25(OH)D_3$ is loosely regulated, mainly dependent upon the amount of substrate [29] and upon negative feedback from $1,25(OH)_2D_3$ [30], whereas the clearance of $25(OH)D_3$ is dependent upon successive hydroxylation in $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$. Both in the LB- and GH-groups, it seems conceivable to suggest a negative feedback on $25(OH)D_3$ production from the high-plasma $1,25(OH)_2D_3$ levels as the clearance of $25(OH)D_3$ is most likely not significantly increased in the LB- and GH-groups versus the control group. The latter is based on (1) the equal $1,25(OH)_2D_3$ production in the LB-group and the increased $1,25(OH)_2D_3$ production in the GH-group, with a negligible contribution to the clearance of $25(OH)D_3$; and (2) on competitive inhibition of the production of $24,25(OH)_2D_3$ mediated by the effect of GH and IGF-I in the LB- and GH-groups and subsequently relatively low-plasma $24,25(OH)_2D_3$ levels versus the control group.

In conclusion, it seems conceivable to suggest that at prepubertal age, GH and/or IGF-I has two action fronts favoring the plasma $1,25(OH)_2D_3$ levels, i.e., decreasing the clearance of $1,25(OH)_2D_3$ under physiological GH excess and increasing the production of $1,25(OH)_2D_3$ under pharmacological GH excess.

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