Vitamin D₃ metabolism in dogs

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Abstract

Plasma concentrations of the main vitamin D₃ metabolites (i.e., 25(OH)D₃, 1,25(OH)₂D₃, and 24,25(OH)₂D₃) were measured in 14 weeks old large- and small-breed dogs (adult body weight 60 kg vs. 6 kg), raised under the same conditions. Levels of 25(OH)D₃ (approx. 22 μg/l) and 1,25(OH)₂D₃ (approx. 40 ng/l) were similar in both groups, whereas plasma 24,25(OH)₂D₃ concentrations were lower in large-breed dogs (7 μg/l vs. 70 μg/l, large- vs. small-breed dogs, respectively). The lower plasma 24,25(OH)₂D₃ concentrations could be explained by the higher plasma GH and IGF-I concentrations in the large- vs. small-breed dogs, and these hormones are known to suppress 24-hydroxylation. Plasma 24,25(OH)₂D₃ concentrations increased during Ca supplementation in small-breed but not in large-breed dogs (100 μg/l vs. 7 μg/l, respectively). Hypophosphatemia induced by a high dietary Ca content was only seen together with increased plasma 1,25(OH)₂D₃ concentrations in euparathyroid dogs and not in hypoparathyroid dogs. Hyperparathyroidism due to Ca deficiency was accompanied by increased plasma 1,25(OH)₂D₃ concentrations and decreased plasma 24,25(OH)₂D₃ concentrations in both large- and small-breed dogs, together with generalized osteoporosis. Large-breed pups fed on a standard diet supplemented with Ca and P had decreased plasma concentrations of both 25(OH)D₃ and 1,25(OH)₂D₃, which may indicate an increased clearance of these metabolites; the low plasma concentrations of the di-hydroxylated vitamin D metabolites were considered responsible for the disturbance in cartilage maturation (i.e., osteochondrosis) in these dogs. Even lower concentrations of all vitamin D₃ metabolites were seen in young dogs raised on a vitamin D₃-deficient diet, which led to disturbances in osteoid and cartilage mineralization (i.e., rickets). These studies indicate that there is a hierarchy of factors regulating vitamin D₃ metabolism in dogs, i.e., GH and IGF-I suppress 24-hydroxylase more than hypercalcemia or hypophosphatemia does; 1,25(OH)₂D₃ and 24,25(OH)₂D₃ are only reciprocally related in hyperparathyroidism; excessive Ca and P intake increases the turnover of vitamin D₃ metabolites; and the synergism between parathyroid hormone and 1,25(OH)₂D₃ seems to play a role in skeletal mineralization. The low plasma 24,25(OH)₂D₃ concentrations in large-breed dogs raised on standard dog food may play a role in the etiology of disturbances in endochondral ossification during the rapid growth phase.

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Keywords: Vitamin D metabolism; Dogs; Nutrition; Growth

1. Introduction

The healing of rickets, a skeletal disease which appeared in epidemic proportions during the urbanization and industrialization of UK in the early 1900s, was first described by Mellanby (1918) in dogs. He fed dogs on oatmeal until they developed clinical and radiological signs of rickets similar to rickets in children: curved bones, thin cortices and enlarged growth plates. The skeletal changes could be prevented and cured by cod liver oil, which contained a substance later known as vitamin D (vitD) (McCollum et al., 1922). An independent study revealed that sunlight could prevent rickets in children (Huldshinsky, 1919) and cure rickets in goats (Steenbock and Hart, 1913). These observations were the impetus for studies on the involvement of vitD in calcium (Ca) metabolism and skeletal development in different species. Amphibians, reptiles, and birds are believed to synthesize sufficient cholecalciferol (vitD₃) in their skin under the influence of ultraviolet light (Holick, 1990). Both herbivores and omnivores synthe-
size vitD₃ in their skin during the summer and utilize ergocalciferol (vitD₃) or vitD₃ from feed the rest of the year (Holick, 1990). However, carnivores such as the domestic dog and cat are solely dependent on oral intake to meet their vitD₃ requirement (How et al., 1994; Morris, 1999). Both natural food and commercially available complete dog food contain sufficient vitD₃ to fulfill a dog’s vitD₃ requirement (Table 1) (NRC, 1974; AAFCO, 2000). Therefore in domesticated dogs rickets is only seen under extreme circumstances, such as a strict vegetarian ration, biliary atresia, and inborn errors of vitD₃ metabolism (Johnson et al., 1988; Kealy et al., 1991; Schulze et al., 2000).

The action of vitD₃ metabolites on the skeleton can be summarized as stimulating the mineralization of newly formed osteoid and cartilage; however, the metabolites are also permissive factors for the stimulation of osteoclasts by parathyroid hormone (PTH; Malluche et al., 1986; Tam et al., 1986). In addition, vitD₃ has a positive influence on Ca homeostasis by influencing active and passive intestinal Ca absorption and renal Ca re-absorption in dogs (Brickman et al., 1973; Pusschett et al., 1972). VitD₃ is metabolized by hydroxylation to both biological active and inactive products. The metabolites of main interest are 25-hydroxycholecalciferol (25(OH)D₃), 1,25-dihydroxycholecalciferol (1,25(OH)₂D₃), and 24,25-dihydroxycholecalciferol (24,25(OH)₂D₃). Hydroxylation of vitD₃ to 25(OH)D₃ is influenced by vitD₃ intake, liver function and plasma 25(OH)D₃ concentration (Rojanasathit and Haddad, 1976; Gascon-Barré and Huet, 1982). The second hydroxylation at the 1α- or 24-location is under the influence of a variety of regulating minerals and hormones including the plasma concentration of Ca, phosphorous (P), PTH, and vitD₃ metabolites (Henry, 1997; Omdahl, 1978) as depicted in Fig. 1.

The pathogenetic role of excessive mineral intake in skeletal diseases has been thoroughly investigated in dogs over the last 30 years (Hedhammar et al., 1974; Hazewinkel et al., 1985; Nap et al., 1993a,b; Goodman et al., 1998; Schoenmakers et al., 2000). Abnormalities in skeletal development related to nutrition are especially seen in dogs with an adult body weight and size close to that of an adult human, e.g., Great Dane dogs. The high growth velocity at young age in these large-breed dogs is known to be associated with transient hypersecretion of growth hormone (GH) and high plasma concentrations of insulin-like growth factor I (IGF-I) (Nap et al., 1992).

In an attempt to elucidate the role of vitD₃ in the maturation and mineralization of newly formed cartilage in large- and small-breed dogs in their fast growth phase, the plasma concentrations of the main metabolites of vitD₃ were measured in dogs with nutritionally induced aberrations of skeletal development. The groups of dogs only differed in their adult body size and/or the nutritional variables vitD₃, Ca, and P.

2. Material and methods

2.1. Diets

All diets were dry dog foods, formulated to meet the recommendations of the US National Research Council’s Nutrient Requirements of Dogs (NRC, 1974), with the following profile: crude protein 20%, crude fat 10%, crude fiber 3.0%, N-free extract 50%, moisture 10% (gram per 100 gram food), with an approximate metabolizable energy content of 15 kJ per g dry matter (DM) provided according to their metabolic energy requirements (Lewis et al., 1987). The mineral content in the control diets was according to the requirements, i.e., 1.1% Ca and 0.9% P (NCaNP diet), but differed from the content of the other diets as indicated below. Each diet contained a vitamin, mineral and trace-element mix including 5000 IU retinyl acetate, 1000 IU vitD₃, and 50 IU dl-α-tocophenol-acetate per kg. Further details of the diet are described elsewhere (Hazewinkel et al., 1991).

2.2. Animals and protocol of the studies

2.2.1. Study I

Five mix-breed dogs (median adult weight (±range) of 20 ±2 kg) were raised immediately after weaning at 6 weeks of age on a semi-synthetic diet without addition of vitD₃ and vitD₂ from 6 till 29 weeks of age. Thereafter they were given a balanced dog food (including 1800 IU vitD₃/kg food).

2.2.2. Study II

Seventeen small-breed dogs (miniature Poodles, median adult weight 6 ±0.5 kg) were divided into three groups at an average age of 8 weeks (range 7–9 weeks) and raised on a diet according to the NRC (1974) requirements but differing in the Ca-content, i.e., 1.1% Ca and 0.9% P (NCaNP; n = 6), 0.05% Ca+0.9% P

Table 1

<table>
<thead>
<tr>
<th>Vitamin D₃ content of ingredients and dog food</th>
<th>Vitamin D₃ (IU/100 g dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver, calf, pork</td>
<td>20–100</td>
</tr>
<tr>
<td>Fish liver oil</td>
<td>5 × 10³–6 × 10⁶</td>
</tr>
<tr>
<td>Butter</td>
<td>10–120</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>150–400</td>
</tr>
<tr>
<td>Milk</td>
<td>0.3–5</td>
</tr>
<tr>
<td>Dog food (Kallfelz, 1989)</td>
<td>40–128</td>
</tr>
<tr>
<td>NRC (1974) requirements</td>
<td>10</td>
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<tr>
<td>AAFCO (2000) requirements</td>
<td>5</td>
</tr>
</tbody>
</table>
2.2.3. Study III

Eleven large-breed dogs (Great Danes, median adult body weight 66 ± 5 kg) were divided into two groups at weaning at 7 weeks of age and raised on a diet according to NRC (1974) requirements but differing in the Ca-content, i.e., NCaNP (n = 6) and 0.55% Ca and 0.9% P (LCaNP; n = 5), respectively.

2.2.4. Study IV

Twenty-four large-breed dogs (Great Danes, median adult body weight 66 ± 5 kg) were divided into three groups at partial weaning at 3 weeks of age and were raised on the following diets that differed in their Ca, or Ca and P content, i.e., NCaNP (n = 9), HCaNP (3.3% Ca and 0.9% P; n = 9), and HCaHP (3.3% Ca and 3.0% P; n = 6).

In study I, the dogs served as their own controls after repletion of the vitD3 content of the food, whereas in studies II–IV, a control group of the same breed and raised on food according to the nutrient requirements of dogs (NRC, 1974, i.e., NCaNP diet) served as a control and reference for that study.

2.3. Measurements in plasma

Blood samples were collected from all dogs after the start of the studies at 14 ± 1 weeks of age, being a representative moment in the period of rapid growth (Hazewinkel et al., 1985; Hazewinkel, 1989; Nap et al., 1992, 1993a, b; Schoenmakers et al., 1998).

Plasma concentrations of Ca and P were determined by colorimetry after complex formation with Arseno-III and by molybdate method with reduction, respectively. PTH was measured with a radioimmunoassay (RIA) for intact human PTH (Nichols Institute Diagnostics, San Juan Capistrano, USA) with an inter-assay coefficient of variation of 5.6% at 38 ng/ml and 6.1% at 277 ng/ml and an intra-assay coefficient of variation of 3.4% at 40 ng/ml and 1.8% at 266 ng/ml (Schoenmakers et al., 1999). The plasma vitD3 metabolites were extracted according to the method described by Bosch et al. (1982). Plasma 25(OH)D3 and 24,25(OH)2D3 concentrations were determined using a competitive protein binding assay, and plasma 1,25(OH)2D3 concentrations...
were measured according to the procedure described by Bouillon et al. (1980), and validated for dogs (Schoenmakers et al., 2000).

In miniature Poodles and Great Danes raised on NCaNP food (Studies II–IV) blood samples were taken 30 and 0 min before feeding and 30, 60, 120 and 180 min after feeding. Plasma GH concentrations were measured in a homologous RIA with intra- and inter-assay coefficient of variation values of 3.8 and 7.2%, respectively (Eigenmann and Eigenmann, 1981). The median of these six individual values was used as the basal value and served to calculate the median value for each group.

Plasma IGF-I concentrations were measured after acid–ethanol extraction in an RIA validated for the dog as described previously (Nap et al., 1993a), using an antiserum (UBK 487) kindly provided by Drs. Underwood and Van Wyk (University of North Carolina, via US National Institute of Diabetes, Digestive and Kidney Diseases). The intra- and inter-assay coefficient of variations were 4.7 and 15.6%, respectively.

### 2.4. Radiographs

Radiographs were made of the right radius and ulna at 15 weeks of age, in a mediolateral view using a conventional diagnostic X-ray system (Maximus M-150, Philips NV, Eindhoven, The Netherlands) on regular black and white films (Cronex 4DDS, DuPont de Nemours GmbH, Frankfurt, Germany). Exposure settings ranged from 48 to 54 kVp and 10–16 mAs, depending on the size of the object. All radiographs were evaluated systematically (Schoenmakers et al., 2000), and changes in the shape and delineation of the growth plates, and density of metaphyseal and cortical bone were evaluated.

### 2.5. Histology

The costochondral junctions of the right 9th rib were surgically resected in the miniature Poodles at 13 weeks of age and in the Great Danes at 16 weeks of age. Microscopic studies on non-decalcified sections were carried out after Mason Goldner staining. The sections were studied for abnormalities in endochondral ossification, the width of the growth plate, and the presence of unmineralized osteoid seams along the trabeculae in the metaphyseal area (Goedegebuure and Hazewinkel, 1986; Schoenmakers et al., 2000).

### 2.6. Statistical analysis

Differences between groups of studies I and III were analyzed by the two-tailed Student’s t-test after testing for homogeneity of variance with Levene’s test, whereas differences between groups in studies II and IV were analyzed by multiple comparisons (Tukey) in a one-way ANOVA. Correlation was calculated with the Spearman’s rank test. Values are given as means±S.E.M.; P < 0.05 was considered to be significant.

### 3. Results

Plasma concentrations of Ca, P, PTH, GH, IGF-I, 25(OH)D3, 24,25(OH)2D3, and 1,25(OH)2D3 were determined in large- and small-breed dogs raised under identical conditions on diets only differing in one or two constituents (i.e., vitD3, or Ca and/or P), thus allowing for comparison of the findings.

Dogs raised on semi-synthetic dry dog food without vitD3 added to the original ingredients had low plasma 25(OH)D3 and 24,25(OH)2D3 concentrations and low normal plasma 1,25(OH)2D3 concentrations compared with those measured after repletion of dietary vitD3 (Table 2, study I).

In the small-breed dogs (study II), basal plasma GH concentrations did not change during the first half year of life (Nap et al., 1992) and ranged between 1.8±0.4 and 4.3±1.4 μg/l. They were 2±0.4 μg/l, at 14 weeks of age. Plasma IGF-I concentrations in these dogs decreased significantly from 137±27 μg/l at 12 weeks of age to 81±27 μg/l at 26 weeks of age during the fast growth phase and were 100±27 μg/l at 14 weeks of age. The small-breed dogs (study II) raised on LLCaNP-diet developed hyperparathyroidism with high plasma 1,25(OH)2D3 concentrations and low plasma 24,25(OH)2D3 concentrations. The small-breed dogs raised on a diet with an increased Ca content (HCaNP diet) had plasma PTH and vitD3-metabolite concentrations not significantly different from those of the control dogs raised on NCaNP diet (Table 2).

The large-breed dogs in study III had high mean basal plasma GH concentrations during their first half year of life and levels were significant related to age (r = −0.87, P < 0.001). Plasma GH concentrations decreased from 15.9±2.3 μg/l at 9 weeks of age to 1.0±0.4 μg/l at 26 weeks of age and were 7±0.5 μg/l at 14 weeks of age. The latter value was not different from the value for the Great Danes of group IV, but was significantly higher than in the miniature Poodles at corresponding age. The plasma IGF-I concentrations of Great Danes in group III were not correlated with age, ranging from 449±27 to 251±10 μg/l between 9 and 26 weeks of age and were 449±20 μg/l at 14 weeks. The latter value was not different from the value found for the Great Danes of study IV, but levels were significantly higher during the whole period of fast growth than they were in the same period in the miniature Poodles. Large-breed dogs raised on the LCaNP diet in study III developed alimentary hyperparathyroidism, associated with elevated plasma 1,25(OH)2D3 concentrations and lowered plasma 24,25(OH)2D3 concentrations (Table 2).
large-breed dogs raised on a diet with a high Ca content (HCaNP diet; study IV) developed hypoparathyroidism with plasma 25(OH)D3 concentrations significantly higher than the control dogs of that study, whereas plasma 1,25(OH)2D3 and 24,25(OH)2D3 concentrations did not differ from those of the control dogs of that study (study IV, NCaNP, Table 2). The large-breed dogs raised on the HCaHP-diet developed hyperparathyroidism associated with low plasma 25(OH)D3 and 1,25(OH)2D3 concentrations whereas plasma 24,25(OH)2D3 concentrations were at control levels (study IV, Table 2).

On radiographic examination, all dogs of study I raised on a VitD3-deficient diet, and all large-breed dogs raised on the HCaNP diet (study IV) had poorly mineralized cortices and wide growth plates in comparison to the control images. Histological examination of the bone biopsies revealed poorly mineralized osteoid (Fig. 2) and cartilage (Fig. 3), which is characteristic of rickets. The small-breed dogs raised on the HCaNP diet (study II) and the large-breed dogs raised on the HCaHP diet (study IV) developed well-mineralized diaphyses and osteoid, as seen in radiographic and histological investigations, respectively. However, in the large-breed dogs skeletal development was associated with radiologically and macroscopically visible irregular widened growth plates due to disturbed cartilage maturation and mineralization, i.e., osteochondrosis (Fig. 3). In the small-breed dogs, the HCaNP-diet led only to macroscopically observable irregularities. In the small- and large-breed dogs raised on a diet deficient in Ca (studies II and III, respectively), radiographs and histology slides revealed long bones with small, regular, and well-developed growth plates and diaphyses with a wide medullar cavity and thin cortices, i.e., severe osteoporosis.

4. Discussion

Dogs are fully dependent on their dietary intake of VitD3 since they are not able to synthesize VitD3 in their skin under the influence of ultraviolet light (Hazewinkel, 1989; How et al., 1994). Nevertheless, rickets is rare in dogs, due to the presence of VitD3 in natural foods and commercially available balanced dog foods (Kallfelz and Dzanis, 1989; Kealy et al., 1991). VitD3-metabolites play a crucial role in active Ca absorption and skeletal mineralization, especially when there is an extremely low dietary mineral content (Harrison and Harrison, 1942). In study I, we demonstrated however that young dogs may develop radiological and histological signs of

<table>
<thead>
<tr>
<th>Characteristics of diet</th>
<th>Diet code</th>
<th>Ca (mmol/l)</th>
<th>P (mmol/l)</th>
<th>PTH (ng/l)</th>
<th>25(OH)D3 (µg/l)</th>
<th>24,25(OH)2D3 (µg/l)</th>
<th>1,25(OH)2D3 (ng/l)</th>
<th>Radiology/histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I (MB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VitD3 deficient (1.1% Ca, 0.9% P)</td>
<td>–</td>
<td>2.7 ± 0.1</td>
<td>2.0 ± 0.3</td>
<td>NP</td>
<td>2.5 ± 0.1’</td>
<td>0.5 ± 0.2’</td>
<td>26 ± 2’</td>
<td>Ricketsa</td>
</tr>
<tr>
<td>1,800 IU vitD3 (1.1% Ca, 0.9% P)</td>
<td></td>
<td>2.7 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>NP</td>
<td>20 ± 0.1</td>
<td>13 ± 5</td>
<td>65 ± 12</td>
<td>Normal</td>
</tr>
<tr>
<td>Study II (SB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1% Ca, 0.9% P</td>
<td>NCaNP</td>
<td>2.8 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>20 ± 2</td>
<td>20 ± 2</td>
<td>68 ± 18</td>
<td>38 ± 2</td>
<td>Normal</td>
</tr>
<tr>
<td>0.05% Ca, 0.9% P</td>
<td>LLCaNP</td>
<td>2.7 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>30 ± 1’</td>
<td>31 ± 9</td>
<td>18 ± 12’</td>
<td>198 ± 35’</td>
<td>Osteoporosib</td>
</tr>
<tr>
<td>3.3% Ca, 0.9% P</td>
<td>HCaNP</td>
<td>3.3 ± 0.1’</td>
<td>2.0 ± 0.2’</td>
<td>18 ± 2</td>
<td>42 ± 15</td>
<td>100 ± 15</td>
<td>75 ± 22</td>
<td>Micr. OCDc</td>
</tr>
<tr>
<td>Study III (LB)</td>
<td></td>
<td></td>
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<tr>
<td>1.1% Ca, 0.9% P</td>
<td>NCaNP</td>
<td>2.9 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>20 ± 5</td>
<td>22 ± 4</td>
<td>7 ± 2</td>
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</tr>
<tr>
<td>0.55% Ca, 0.9% P</td>
<td>LCaNP</td>
<td>2.9 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>30 ± 2’</td>
<td>30 ± 10</td>
<td>2.1 ± 0.5’</td>
<td>200 ± 25’</td>
<td>Osteoporosib</td>
</tr>
<tr>
<td>3.3% Ca, 0.9% P</td>
<td>HCaNP</td>
<td>3.9 ± 0.1’</td>
<td>2.3 ± 0.1’</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
<td>9 ± 3</td>
<td>47 ± 1</td>
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<tr>
<td>3.3% Ca, 3.0% P</td>
<td>HCaHP</td>
<td>2.9 ± 0.1</td>
<td>2.4 ± 0.1’</td>
<td>75 ± 30’</td>
<td>10 ± 1’</td>
<td>7 ± 1</td>
<td>29 ± 1’</td>
<td>Macr. OCDd</td>
</tr>
</tbody>
</table>

Small-breed dogs (SB, study II) and large-breed dogs (LB, studies III and IV) were raised on diets with 1000 IU vitamin D3 per kg diet and only differing in the mineral content as indicated (% of dry matter). In medium-size dogs (MB, study I) VitD3-deficient food was given for 29 weeks, followed by VitD3-supplemented food. NP = not performed.

* Rickets: mushroom appearance of physal plates on radiographs and osteoid seams along mineralized bone in histological slides.

b Osteoporosis: severe osteosclerosis of cortices and cancellous bone.

c OCD: focal disturbance of chondrocyte maturation and thus hampered endochondral ossification (Micr.: only noticeable on microscopical investigation; Macr.: visible macroscopically and on radiographs).

* Significantly different (P < 0.05) from control dogs (1.1% Ca, 0.9% P) of that study.

Table 2
Biochemical profiles (mean ± S.E.M.) and skeletal development in dogs raised on diets differing in vitamin D3, or Ca and/or P content at 14 ± 1 weeks of age.
rickets even though the mineral content of their food meets the requirements (NRC, 1974), and plasma Ca and P concentrations do not differ from control values. The defective mineralization of both newly formed osteoid and cartilage limits the loss of extracellular Ca, thus keeping plasma Ca concentrations within biological safe margins under the influence of high plasma PTH concentrations.

One of the most striking findings in this study was the 10-fold higher plasma 24,25(OH)2D3 concentrations in small-breed dogs than in large-breed dogs. This can be related to the high plasma concentrations of growth factors in the large-breed dogs, a condition characterized as transient juvenile gigantism by Nap et al. (1992). The findings of this study are consistent with this, because Great Danes and miniature Poodles at 14 weeks

Fig. 2. Histological section (Mason Goldner's Trichrome, 25 × ) of the metaphyseal area of the 9th rib of two Great Danes at 15 weeks of age, raised on food containing 1.1% Ca and 0.9% P (NCaNP; top) or 3.3% Ca and 0.9% P (HCaNP; bottom). In the latter, unmineralized osteoid seams were abundantly present along the trabeculae (indicated with O).
of age had basal plasma GH concentrations of 7 and 2 µg/l, respectively, and plasma IGF-I concentrations of 449 and 100 µg/l, respectively. Although circulating levels of vitD$_3$ metabolites are a function of synthesis, use, and further metabolism, there is in vivo (Goff et al., 1990; Lund et al., 1981) and in vitro (Condamine et al., 1994) evidence that GH influences the hydroxylation of 25(OH)D$_3$ either directly or indirectly via IGF-I. The administration of GH to children with GH deficiency and to normal piglets increases circulating plasma 1,25(OH)$_2$D$_3$ concentrations, whereas plasma 24,25(OH)$_2$D$_3$ concentrations decrease (Wei et al., 1996; Goff et al., 1990). Accordingly, in acromegalic patients with high plasma GH and IGF-I concentrations, plasma concentrations of 1,25(OH)$_2$D$_3$ are increased (Lund et al., 1981). IGF-I increases 1,25(OH)$_2$D$_3$ synthesis in mammalian kidney cells especially under hypophosphatemic conditions (Condamine et al., 1994). Although the vitD$_3$ content of the diets in all studies with the miniature Poodles and Great Danes met the NRC (1974) requirements for young dogs, a higher requirement in the fast growing dogs of large breeds can be anticipated. In large-breed dogs, a high demand for Ca for the growing skeleton and the high GH and IGF-I levels may favor the synthesis of 1,25(OH)$_2$D$_3$ at the expense of 24,25(OH)$_2$D$_3$. This may be even more so if the synthesis of 1α-hydroxylase is stimulated by hyperparathyroidism, as in the dogs on the LCaNP diet (study III). Additional studies of large-breed dogs raised on food supplemented with vitD$_3$ are warranted to elucidate the cause of the significantly lower plasma 24,25(OH)$_2$D$_3$ concentrations in large-breed dogs compared with small-breed dogs when both are raised on a diet with the same vitD$_3$ content.

Large- and small-breed dogs with dietary Ca deficiency had significantly increased plasma 1,25(OH)$_2$D$_3$ concentrations together with hyperparathyroidism. Because of a decreased Ca intake, hyperparathyroidism develops, resulting in the stimulation of the 1α-hydroxylase and thus increased 1,25(OH)$_2$D$_3$ synthesis. If there is a negative Ca balance, 1α-hydroxylation occurs at the expense of 24-hydroxylation (Henry, 1997). Active Ca absorption is stimulated by 1,25(OH)$_2$D$_3$ by the induction of Ca-binding proteins in intestinal cells, which increases the efficiency of Ca absorption (Wasserman and Fullmer, 1995). Metabolic studies with $^{45}$Ca as a tracer have revealed that the fraction of Ca absorbed from food increases from approximately 45% in dogs raised on diet containing 1.1% Ca to more than 95% in large- or small-breed pups raised on a Ca-deficient diet (with 0.55 and 0.05% Ca, respectively) (Hazewinkel et al., 1991; Nap et al., 1992). Despite the increase in fractional Ca absorption, severe osteoporosis was seen in both large- and small-breed dogs raised on a Ca-deficient diet due to hyperparathyroidism which increased osteoclasia (Malluche et al., 1986).
Dogs raised on food containing excess Ca (HCaN; studies II and IV) developed hypophosphatemia as a result of increased P sequestration in the intestinal lumen and in the skeleton (Nap et al., 1992; Schoenmakers et al., 2000). Hypercalcemia due to a high Ca intake at an early age (i.e., 3 weeks of age) suppresses PTH secretion and chief-cell mitosis, causing severe hypoparathyroidism (Schoenmakers et al., 2000). The large-breed dogs with severe hypoparathyroidism had hypercalcemia and hypophosphatemia, whereas plasma 1,25(OH)2D3 concentrations remained at control levels. Hypophosphatemia is a strong stimulus for 1α-hydroxylase activity (Baxter and DeLuca, 1976; Tanaka and De Luca, 1973), especially in combination with high IGF-I levels (Condamine et al., 1994). However, in the large-breed dogs raised on the HCaNP diet (study IV), there was no increase in plasma 1,25(OH)2D3 concentrations, which may be ascribed to the attenuated anabolic effect of the very low plasma PTH concentrations on 1α-hydroxylase activity, as was also reported in hypoparathyroidism in humans (Haussler et al., 1976) and after parathyroidectomy in animals (Garabedian et al., 1972). The decreased conversion of 25(OH)D3 to 1,25(OH)2D3 is reflected by the high plasma 25(OH)D3 concentrations compared to those of the control group. Unlike the large-breed dogs, the small-breed dogs raised on the HCaNP diet (study II), which they started at an older age, had normal plasma PTH concentrations. The severe hypophosphatemia may have tended to increase plasma 1,25(OH)2D3 concentrations in the small-breed dogs raised on the HCaNP diet, even under hypercalcemic conditions. From the above, we conclude that adequate PTH secretion is an important factor for the stimulation of 1α-hydroxylase activity under hypophosphatemic conditions.

Small-breed dogs raised on control food supplemented with Ca (i.e., HCaNP diet) had increased plasma 24,25(OH)2D3 concentrations compared with the control group. This can be explained by the hypercalcemia and the hypophosphatemia-induced increase in plasma 1,25(OH)2D3 concentrations, since both stimulate 24-hydroxylase activity (Shinti et al., 1992). It is remarkable that plasma 24,25(OH)2D3 concentrations were not increased in the large-breed dogs raised on the same HCaNP diet even though they were hypercalcemic and hypophosphatemic. This may be explained by the following mechanisms: (1) unchanged 24-hydroxylase activity due to the blunted increase in 1,25(OH)2D3 production, which is the main positive regulator of 24-hydroxylase activity under hypercalcemic conditions (Shinti et al., 1992), (2) high plasma GH and IGF-I concentrations and subsequent increased 1α-hydroxylation and suppressed 24-hydroxylation of 25(OH)D3, (3) insufficient vitD3 intake to supply 25(OH)D3 for 24-hydroxylation, (4) increased metabolic clearance of 24,25(OH)2D3, and (5) a combination of these mechanisms. Further studies of small-breed dogs with exogenous GH administration and of large-breed dogs raised on food with extra vitD3 may indicate which of these possible explanations is the most likely.

There were marked differences in skeletal changes between young dogs of the large and small breeds when the pups were raised on the HCaNP diet. The small-breed dogs did not develop physical or radiological skeletal abnormalities on this diet: histological examination of the physeal plates revealed only slight microscopic disturbance in endochondral ossification. In contrast, the large-breed dogs had physical, radiological, and histological signs of poor skeletal mineralization with wide growth plates and poorly mineralized osteoid, i.e., rickets. Hypophosphatemia in the small-breed pups did not affect skeletal mineralization and thus may not be solely responsible for the occurrence of rickets in the large-breed dogs. It can be anticipated that 1,25(OH)2D3 synthesis is suppressed in the large-breed dogs due to severe hypoparathyroidism although this is not reflected in the plasma 1,25(OH)2D3 concentrations. The relative deficiency in 1,25(OH)2D3 in the HCaNP group of large-breed dogs is reflected by the presence of radiological and histological signs of rickets (Figs. 2 and 3), a decreased fractional Ca and P absorption in the intestine, as published by Schoenmakers et al. (1999) for this group of dogs, and a decreased metabolism of 25(OH)D3, resulting in significantly higher plasma 25(OH)D3 concentrations compared with those of the control group. In addition to 1,25(OH)2D3, 24,25(OH)2D3 is currently also considered to be an important factor for optimal skeletal development and mineralization (Boyan et al., 2001). Elevated plasma 24,25(OH)2D3 levels are associated with stimulated differentiation and maturation of chondrocytes (Schwartz et al., 1995) and increased bone mineralization (Ono et al., 1996). Synergism between PTH and 1,25(OH)2D3 induces the expression of 24-hydroxylase, which results in increased 24,25(OH)2D3 synthesis and associated mineralization of the skeleton, thereby counter-regulating PTH-induced osteoclasia (Ono et al., 1996), as probably occurred in the small-breed dogs raised on the HCaNP diet (study II). In contrast, plasma PTH concentrations decreased considerably in the large-breed dogs raised on the HCaNP diet (study IV) without an increase in plasma 24,25(OH)2D3 concentrations. Therefore, the severe disturbances in cartilage and osteoid mineralization in the large-breed dogs raised on food supplemented with Ca can be ascribed to hypophosphatemia and/or the absence of a synergistic effect between PTH and 1,25(OH)2D3 to stimulate 24-hydroxylase synthesis. Further studies of large-breed dogs with nutritional hypoparathyroidism supplemented with exogenous 1,25(OH)2D3 or 24,25(OH)2D3 may reveal the role of these metabolites in skeletal development.
In a previous study (Schoenmakers et al., 1999), large-breed dogs raised on the HCaHP diet had a threefold higher intestinal absorption and increased bone accretion of Ca and P than control dogs raised on the NCaNP diet (study IV). In immature animals, Ca is absorbed via a concentration-driven passive mechanism (Charles, 1992). The supra-positive Ca balance in the dogs raised on the HCaHP diet resulted in decreased plasma 1,25(OH)2D3 concentrations. Since the dogs of study IV were eating the same amount of food per kg0.75 body weight with the same vitD3 content as the dogs on the NCaNP diet, it may be assumed that 25(OH)D3 was synthesized at the same rate in dogs raised on the HCaHP and NCaNP diets. The low plasma 25(OH)D3 and 1,25(OH)2D3 concentrations in the dogs raised on the HCaHP diet reflect a high clearance of both vitD3 metabolites (Halloran et al., 1986) rather than vitD3 deficiency, as was apparent in the dogs of study I. Low plasma 1,25(OH)2D3 concentrations protect against further, active absorption of Ca and P (Schoenmakers et al., 1999). In the dogs raised on the HCaHP diet, normocalcemia was maintained in combination with mild hypophosphatemia, by increased sequestration of P in intestine and bone; by decreased activity of 1,25(OH)2D3 (Sutton et al., 1976; Schoenmakers et al., 1999), and by decreased maximum tubular absorption of P (Sutton et al., 1976; Schoenmakers et al., 1999). The elevated plasma PTH concentrations may be explained by the attenuated negative feedback of 1,25(OH)2D3 on the chief cells of the parathyroid gland (Russel et al., 1986). The dogs raised on the HCaHP diet had well-mineralized cortices and cancellous bone, but disturbed endochondral ossification, which is characteristic of osteochondrosis (Hedhammar et al., 1974; Hazewinkel et al., 1985). Osteochondrosis in the canine species, only seen in young, fast growing animals of large breeds is characterized by a disturbance of cartilage maturation and subsequently delayed cartilage mineralization. Cartilage cell differentiation and matrix mineralization are influenced by 24,25(OH)2D3 and 1,25(OH)2D3, as has been demonstrated in in vitro studies (Plancho et al., 1982). The physiological balance between 24,25(OH)2D3 and 1,25(OH)2D3 is a prerequisite for undisturbed endochondral ossification (Boyan et al., 2001). The disturbed balance between these metabolites may be the etiological background for the severe osteochondrosis seen in the large-breed dogs with a high mineral intake and decreased plasma 1,25(OH)2D3 concentrations and relatively low plasma 24,25(OH)2D3 concentrations. Further studies of growing large- and small-breed dogs with 24,25(OH)2D3 supplementation or blockade of hydroxylation of the C24 position, respectively, may elucidate the role of 24,25(OH)2D3 in endochondral ossification.

In this study, we demonstrated that in growing dogs irrespective of the growth rate there is only a reciprocal relationship between plasma 1,25(OH)2D3 and 24,25(OH)2D3 concentrations during hyperparathyroidism. Hypophosphatemia induced by high Ca intake will only lead to increased plasma 1,25(OH)2D3 concentrations in the presence of PTH. Excessive dietary intake of Ca and P is associated with low plasma 25(OH)D3 and 1,25(OH)2D3 concentrations due to increased clearance of both metabolites. Decreased plasma 1,25(OH)2D3 concentrations in combination with low plasma 24,25(OH)2D3 concentrations may influence endochondral ossification in large-breed dogs. Large-breed dogs with high plasma GH and IGF-I concentrations have lower plasma 24,25(OH)2D3 concentrations than small-breed dogs raised on the same food do. Further studies of vitamin D metabolism in genetic subgroups of dogs with different growth rates may reveal the influence of vitD3 metabolites on skeletal development.

References


Shinti, T., Jin, Ch., Nishimura, A., Nagai, Y., Ohyama, Y., Noshiero, M., Okuda, K., Suda, T., 1992. Parathyroid hormone inhibits 25-


