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# CALCIUM METABOLISM: AN OVERVIEW OF ITS HORMONAL REGULATION AND INTERRELATION WITH SKELETAL INTEGRITY

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## INTRODUCTION

Lameness due to disturbances in skeletal development is frequently diagnosed in companion animal practice. These disturbances include decreases in skeletal modelling (enostosis, canine wobbler syndrome), increases in bone resorption (with pathological fractures as a result), and disturbances in endochondral ossification (osteochondrosis and hypovitaminosis D). Especially decreased modeling and osteochondrosis are seen frequently in dogs of certain large breeds, including Great Danes (13, 14, 31, 33, 37). The severity of clinical signs can be aggravated by excessive intake of food and/or Ca (15, 21, 24). The role of high protein diets in the etiology of osteochondrosis in dogs was rebutted by Nap *et al* (1991). Other nutritional factors were suggested to play a role in the etiology but were not investigated (29), leaving calcium (Ca) as the main causative dietary factor of this disease.

Very sensitive regulation mechanisms have evolved to keep plasma Ca concentration constant in an environment that is deficient in Ca (3). Effective mechanisms to prevent the negative effects of excessive Ca uptake and absorption are less well developed.

In this article an overview will be given of Ca regulation in dogs and the interrelation with skeletal growth and mineralization, illustrated with relevant clinical information.

## REGULATION OF PLASMA CALCIUM CONCENTRATION

Ca is the most abundant mineral in vertebrates and has many important physiological functions (4, 20, 23). As a component of the hydroxyapatite crystal, it contributes to the solidity of the skeleton. Only a small fraction of total body Ca is present outside the skeleton, in soft tissues and extracellular fluid. The extracellular Ca is vital for nerve conduction, muscle contraction, and enzymic activity, and as a cofactor in the blood coagulation cascade. For these processes an optimal Ca concentration is crucial. It is therefore of vital importance to maintain the extracellular Ca concentration within narrow limits (4, 8). The skeleton serves as a reservoir of minerals in the extracellular fluid (7, 23). When the extracellular Ca concentration is challenged, maintenance of skeletal integrity is sacrificed to the necessity for deposition of Ca in or mobilization of Ca from the skeleton in order to keep plasma Ca concentration constant within narrow limits (17, 30, 34).

The extracellular Ca concentration is maintained at its optimal concentration by physicochemically-driven processes, the concerted actions of the calciotropic hormones, and control by Ca sensors. Ca concentration varies little in the individual. It is normally between 2.0 and 3.0 mmol/l in the adult dog and may be slightly higher in young dogs. Approximately 40% of the plasma Ca is loosely bound to plasma proteins (mainly albumin) and another 10% is tightly bound in complexes with other ions. The remaining 50% of plasma Ca circulates as ionized Ca ($Ca^{2+}$) and is the fraction that is vital in biological processes (23). The extracellular Ca concentration is constantly challenged by Ca absorption from and secretion into the intestine, by Ca resorption from and accretion in bone, and by Ca loss mainly via the kidney (2, 4, 20). During pregnancy and lactation, both the fetus and the mammary gland also withdraw Ca from the maternal plasma (2). When Ca metabolism is chronically challenged by persistently high or low Ca absorption (10, 17) or by primary hyperparathyroidism (1, 6), the plasma Ca concentration is still continuously tightly regulated, although this may be at a level different from normal (1, 6, 10). The Ca concentration is then constantly maintained at a level outside the reference range (1, 6, 10) as will be discussed below.

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## PLASMA CALCIUM CONCENTRATION AND THE CALCIOTROPIC HORMONES

In this section the synthesis, secretion, and actions of parathyroid hormone, calcitonin, and VitD will be reviewed.

### *Parathyroid hormone (PTH)*

*Synthesis:* The precursor pre-pro-PTH is produced within the chief cells of the parathyroid glands. Its synthesis is regulated by the extracellular Ca concentration, VitD metabolites, and possibly CT. The post-transcriptional cleavage of PTH from its precursors occurs in two steps, first to pro-PTH, and then to biologically active PTH, a protein consisting of 84 amino acids (i.e., intact PTH (iPTH). iPTH is stored in secretory granules in the chief cells (23, 36). In normo- and hypercalcemic conditions, the secretion of the C- terminal (35-84) PTH fragment predominates, whereas predominantly iPTH is secreted during hypocalcemia (11).

*Secretion and actions*: A decrease in plasma Ca concentration leads to the release of iPTH from its storage granules into the circulation (Figure 3) (1, 6). The plasma Ca concentration increases as a result of dissociation of Ca ions from their binding components in plasma and Ca release from intracellular stores and the loosely bound Ca pool in bone (3), together with an increase in Ca reabsorption in the kidney. These changes increase plasma Ca concentration acutely (within 1 minute) (4, 12). If the decrease in extracellular Ca concentration continues, PTH induces increased osteoclasia, which enhances Ca liberation within hours. In addition, PTH stimulates the renal production of $1,25(OH)_2$VitD, which stimulates the active absorption of Ca in the intestine, acts as a permissive factor for PTH-mediated bone resorption, and influences the recruitment of the osteoclasts from the osteoprogenitor cell lineage. These changes enhance the liberation of Ca in the extracellular fluid on a long-term basis (days to weeks) (23). This is illustrated by the occurrence hyperparathyroidism together with increased plasma concentrations of $1,25(OH)_2$VitD in miniature poodles raised on food with a low Ca content (Figure 1). In these dogs, the efficiency of intestinal absorption was doubled and skeletal demineralization

was increased ten-fold over that in dogs raised on a high-Ca diet (28, 30).

An increase in plasma Ca concentration leads to the suppression of iPTH secretion. The plasma Ca concentration then decreases as a result of increased Ca excretion in the urine and cessation of bone resorption, both within minutes, and decreased $1,25(OH)_2$VitD production over a longer interval. Chronic intake of a diet with a high Ca content results in diminished PTH production and secretion and thus to decreased osteoclastic activity (34).

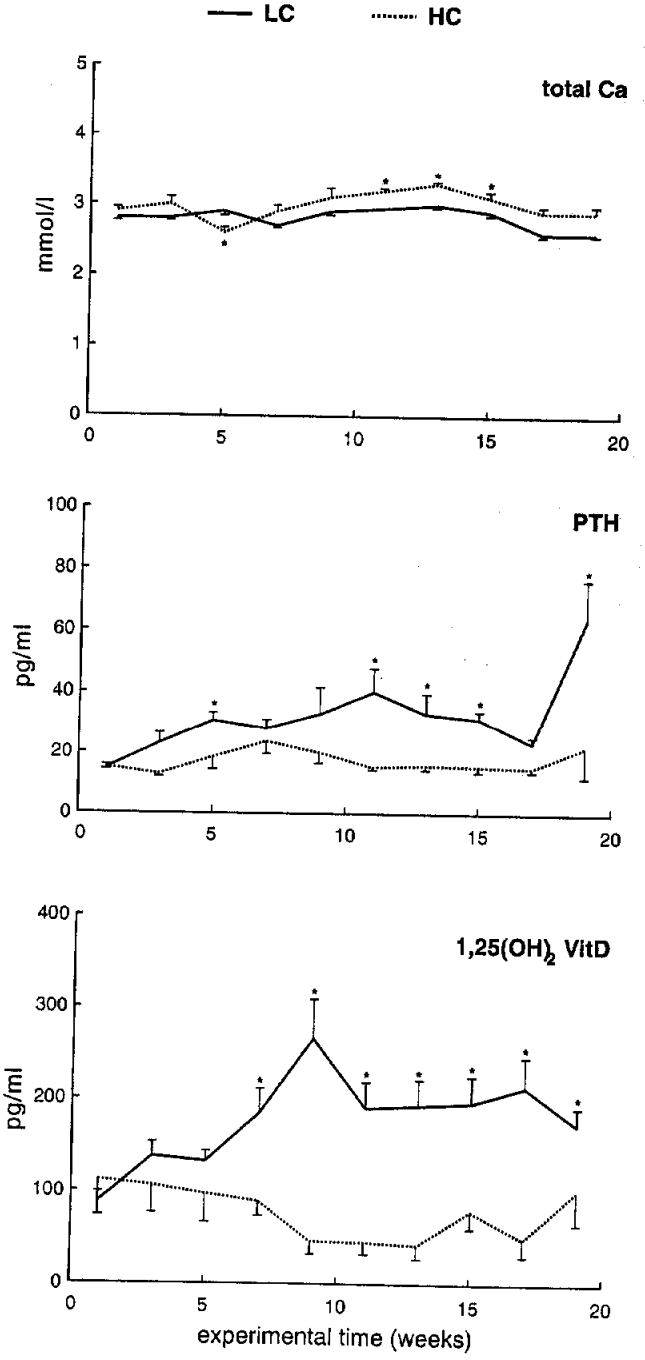



Figure 1. Plasma concentrations (mean ± SEM) of total calcium (Ca), parathyroid hormone (PTH), and 1,25 dihydroxycholecalciferol ($1,25[OH]_2$VitD) in a group of miniature poodles raised on a food with a high Ca (3.3% Ca on dry matter base (dmb) (HC), n=5) or a low Ca (0.05% on dmb, (LC), n=6) content starting at the age of 8 weeks (30).

***Calcitonin (CT)***

*Synthesis:* CT is synthesized in the parafollicular or C-cells, mainly located in the thyroid glands. As in PTH synthesis, gene transcription results in a larger precursor molecule, preprocalcitonin (pre-proCT), which is processed within the C-cell (3). CT and proCT are stored in secretory vesicles in the C-cells (3). C-cell hyperplasia has been reported to be induced by chronic hypercalcemia (15). The synthesis of CT is inhibited by $1,25(OH)_2$VitD and possibly by many other, yet undefined, factors (3).

*Secretion and actions*: The principal regulator of CT secretion is the plasma ionized Ca concentration. Upon a sudden rise in the plasma $Ca^{2+}$ concentration, CT is secreted from the granules into the circulation, and it lowers the plasma $Ca^{2+}$ concentration by its immediate inhibiting effect on bone resorption, by promotion of osteoblastic activity, by deposition of Ca within mitochondria, and by increasing renal Ca excretion. In addition, CT decreases the rate of gastric emptying and food intake, thus reducing the absorption of Ca from the gastrointestinal tract (3).

At low plasma $Ca^{2+}$ concentrations, CT secretion is inhibited to allow for a rise in plasma $Ca^{2+}$ concentration under the influence of PTH (3). The role of CT in Ca homeostasis appears to decrease with increasing age in dogs, resulting in low basal plasma CT concentrations with increasing age (Figure 2) (34). Especially at an early age, but also in other conditions of high Ca demand such as in pregnancy and lactation, the actions of CT are directed at avoiding hypercalcemia and consequently renal loss of Ca. CT enhances the deposition of Ca in bone and in the exchangeable Ca pool, and prevents excessive bone resorption by decreasing osteoclastic function (3). In addition, CT plays an important role in sustaining the concentration of $1,25(OH)_2$VitD independent of PTH (3, 23).

Because of poor cross-reactivity of canine CT with antisera raised against CT of other species (27), a homologous RIA for canine CT was developed for the determination of canine plasma CT concentration with high precision (Figure 2) (34).

***Vitamin D (VitD)***

*Synthesis:* Unlike man, the dog is dependent on dietary sources of VitD, since cutaneous synthesis of the vitamin is inadequate in the dog, due to the low concentration of the VitD precursor 7-dehydrocholesterol in the skin of carnivores (18, 22). VitD is converted in the liver to 25(OH)VitD or is stored in the liver and adipose tissue for later use (23). 25(OH)VitD

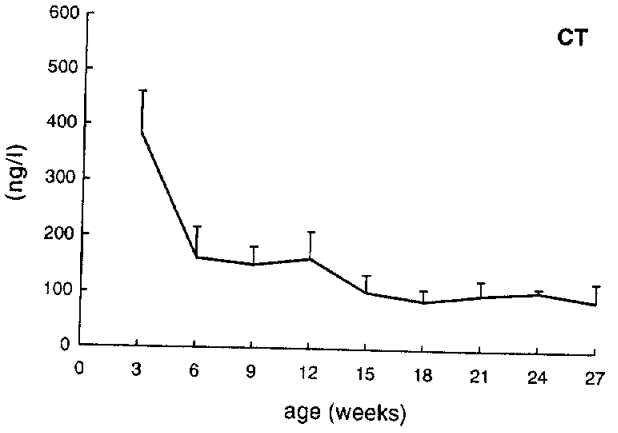



Figure 2. The plasma concentration (mean ± SEM) of calcitonin (CT) in a group of young Great Danes (n= 9) decreases with age.

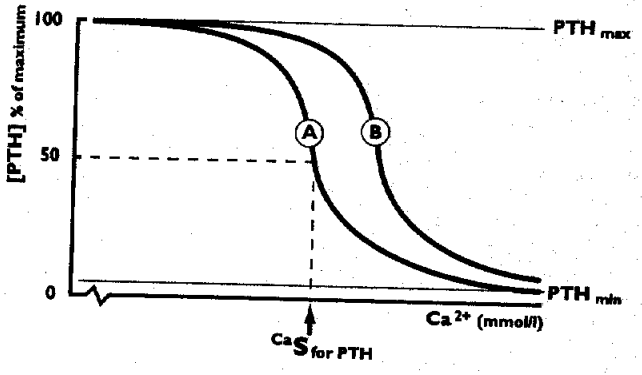



Figure 3. The relation between the extracellular ionized calcium ($Ca^{2+}$) concentration and parathyroid hormone (PTH) plasma concentration expressed as % of its maximal value after stimulation, in normal individuals (A) and in case of primary hyperparathyroidism (B). In the latter, PTH secretion is already stimulated in the hypercalcemic range, shifting the curve to the right. The extracellular $Ca^{2+}$ concentration, suppressing the plasma PTH concentration to 50% of its maximum (the $Ca^{2+}$ setpoint for PTH secretion ($^{Ca}S_{for\ PTH}$)) is consequently higher in this group of patients (34, modified from 8).

is converted by 1α-hydroxylase in the kidney to the most active metabolite, $1,25(OH)_2$VitD, or alternatively to $24,25(OH)_2$VitD by 24 hydroxylase, present in many tissues (23). The hydroxylation of 25(OH)VitD to $1,25(OH)_2$VitD is stimulated by low plasma concentrations of Ca or P and by high PTH concentrations, as well as by CT, insulin-like growth factor-1 (IGF-1) and/or growth hormone (GH) (23). The major part of circulating VitD and its metabolites is bound to VitD binding proteins and other plasma proteins, which transport VitD and its metabolites to their target tissues and regulates their cellular ingress (23).

*Actions:* In the intestine $1,25(OH)_2$VitD stimulates the active absorption of Ca and P. In the kidney $1,25(OH)_2$VitD stimulates the reabsorption of Ca and P from the glomerular filtrate (section 4.2). In growing cartilage $1,25(OH)_2$VitD and $24,25(OH)_2$VitD influence the processes of cartilage maturation and calcification (23). In calcified bone $1,25(OH)_2$VitD acts as a regulator of bone resorption (23).

## CALCIUM SENSING AND THE SETPOINT FOR EXTRACELLULAR CALCIUM

### *Calcium sensing*

Sensing of the extracellular $Ca^{2+}$ concentration is essential for the maintenance of its constancy (8). Changes in its concentration are sensed by Ca receptors (CaR). These extracellular receptors are present on all cells and organs having a significant role in Ca homeostasis, i.e., the chief cells of the parathyroid, the C-cells, the kidney, osteoclasts, osteoblasts, the placenta, and the intestine. Some organs not directly involved in Ca homeostasis, such as the brain and the lung, also contain CaRs. Presumably different second messenger systems are involved in the events following the sensing of changes in the extracellular Ca concentration, explaining the divergent responses of the different tissues involved in the maintenance of the extracellular Ca concentration.

Factors regulating the number of CaRs are largely unidentified (5, 8).

### *The setpoint for extracellular calcium*

Plasma $Ca^{2+}$ concentration and PTH and CT secretion are related in a curvilinear mode (1, 6). PTH secretion is stimulated at low $Ca^{2+}$ concentrations until a plateau in plasma PTH concentration is reached, and is inhibited at high $Ca^{2+}$ concentrations (Figure 3) (1, 6). For CT the relationship is the inverse of this (3). Only over a narrow range of plasma $Ca^{2+}$ concentrations there is a linear segment in the sigmoid relation between PTH and $Ca^{2+}$. The linear segment includes the $Ca^{2+}$ concentration that inhibits PTH secretion to 50% of its maximum value. This value of $Ca^{2+}$ is defined as the $Ca^{2+}$ setpoint for PTH secretion ($^{Ca}S_{for\ PTH}$) (Figure 3) and is close to the concentration of $Ca^{2+}$ that is maintained under nonstimulated conditions (1, 6). Alterations in the $^{Ca}S_{for\ PTH}$ occur under several conditions, such as in primary hyperparathyroidism in man (pHPT) (6) and alimentary secondary hyperparathyroidism (sHPT) in dogs induced by a diet deficient in both Ca and VitD (Figure 3) (10). The significance of an altered $^{Ca}S_{for\ PTH}$ for the developing skeleton and the maintenance of skeletal integrity in adulthood remains to be resolved.

## THE ROLE OF THE INTESTINE, KIDNEY, AND BONE IN CALCIUM METABOLISM

### *The intestine*

Ca absorption is the sum of passive and active transfer of this mineral through the intestinal wall (9). At an early age the passive, paracellular absorption prevails and is dependent on the concentration gradient for Ca between the intestinal lumen and the interstitium. In mature dogs the greater part is absorbed by active, transcellular absorption, mediated by $1,25(OH)_2$VitD (Figure 4). This implies that at a young age, Ca absorption is high when the dietary intake of Ca is high (17, 21, 30, 34).

Small quantities of endogenous Ca are lost into the gastrointestinal tract by passive diffusion in digestive secretions and as a result of the obligatory cell death of intestinal mucosa cells. Part of the lost endogenous Ca is reabsorbed in the lower part of the intestinal tract. This loss of Ca is more relevant in dogs of small breeds than in those of large breeds at a young age. In dogs of small and large breeds fed the same diet with 1.1% Ca on a dry matter basis, this loss was 53% and 10%,respectively, of the amount of Ca absorbed from the intestine (30, 34).

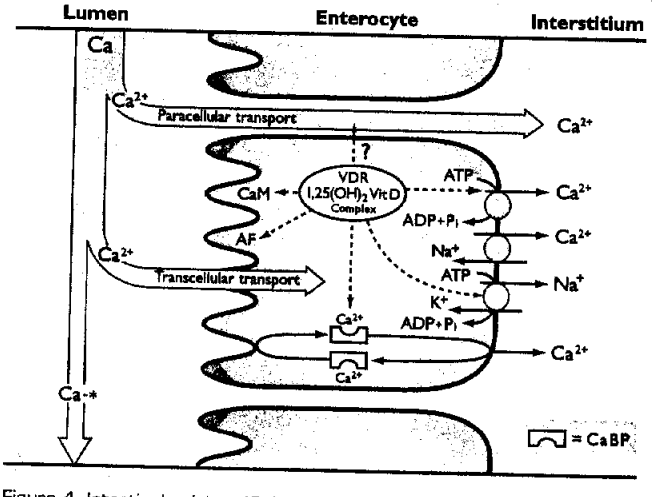



Figure 4. Intestinal calcium (Ca) absorption includes paracellular diffusion and active transcellular transport. The latter is augmented by 1,25 dihydroxycholecalciferol ($1,25\ (OH)_2$VitD) after binding on the vitD receptor (VDR), stimulating the production of proteins, i.e., Ca binding-protein (CaBP), alkaline phosphatase (AF), and calmodulin (CaM), which enhance the uptake of Ca in the enterocytes, and the expulsion of Ca at the serosal side by an energy-dependent pump. Part of the dietary Ca leaves the intestine unabsorbed (Ca-*) (34)

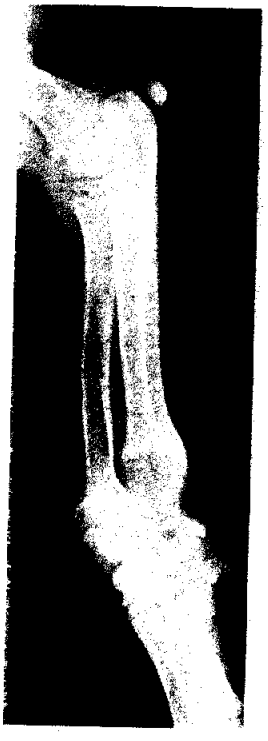
Figure 5. A young Border collie with atresia of the ductus choledochus and an overfilled gall bladder (g) developed severe hyperbilirubinemia and disturbance of skeletal mineralisation (below), the latter due to defective fat and Vitamin D absorption (courtesy F.J. van Sluys and W.Th.C. Wolvekamp).

### *The kidney*

The kidney plays an important role in maintaining a constant plasma Ca concentration. In addition to the non-hormonal regulation based on the concentration gradient across the glomerular membrane, there is modulation of renal Ca handling by the calciotropic hormones (25). PTH is central in this function, regulating renal reabsorption of Ca and P and stimulating $1,25(OH)_2$VitD synthesis. Furthermore, the kidney plays an important role in the degradation and clearance of the calciotropic hormones (3).

### *The bone*

Bone is a dynamic tissue, in both young and adult animals. Bone is constantly resorbed and then replaced by new bone. This aspect of bone biology allows for the adaptation of bone to its mechanical demands (bone remodeling) and the maintenance of the characteristic shape of the bones during longitudinal bone growth (bone modeling). The latter includes bone resorption at the endosteal and metaphyseal sides and ensures the proportional widening of the foramina to allow enlarging soft tissues to pass through (19).



The processes of osteoid deposition and mineralization and bone resorption are highly coordinated in healthy bone, resulting in an increase in bone mass in growing animals and little or no net loss of bone mass in adults. The regulation of these processes is complex and only partly elucidated (12, 23). The osteoblasts produce a thin layer of osteoid on the remaining bone surface. The osteoid then undergoes a variety of biochemical changes before it can be mineralized by the deposition of hydroxyapatite (12). Osteoclasts are only able to resorb mineralized osteoid and do so to model and remodel the skeleton to its mechanical needs. In addition, bone resorption and the deposition of Ca and P in bone play an important role in the maintenance of a constant extracellular Ca concentration (12).

### *Regulation of bone metabolism by the calciotropic hormones*

The calciotropic hormones influence osteoclastogenesis, osteoclastic and osteoblastic activity, and osteoid maturation and calcification (12, 23).

**PTH** enhances the activity and life span of the existing osteoclasts and increases the number of osteoclasts by its influence on their differentiation from the hematopoietic cell lineage. At the same time, PTH inhibits osteoblastic activity and differentiation (3, 23). PTH stimulates the differentiation and proliferation of chondrocytes, matrix maturation, and possibly mineralization (35).

The effects of **CT** on osteoclasia are opposite to those of PTH; CT inhibits osteoclastic motility and resorbing activity. After longer exposure to elevated CT concentrations the number of osteoclasts decreases, although chronic exposure of osteoclasts to elevated CT concentrations leads to decreased osteoclastic sensitivity to CT (known as the escape phenomenon) (3). CT stimulates bone formation by the osteoblasts, either directly or via the osteoclasts (3). The limited data available on the role of CT in the process of endochondral ossification, suggests that CT stimulates chondrogenesis and mineralization of the physeal matrix (3). From experimental studies it appears that hypercalcitoninemia may be responsible for the delay of chondrocyte maturation and thus endochondral ossification (15, 21).

**VitD metabolites** exert their actions on bone directly, or indirectly via local factors produced by bone cells (39). The actions of $1,25(OH)_2$VitD on bone promote bone resorption, as well as bone formation and mineralization. The action depends on the duration of exposure of bone tissue to elevated plasma $1,25(OH)_2$VitD concentrations. $1,25(OH)_2$VitD stimulates the differentiation of osteoclasts and osteoblasts from their precursor cells. In the existing osteoblast, the actions of $1,25(OH)_2$VitD promote the calcification of osteoid already present; the production of alkaline phosphatase, osteopontin, osteocalcin, and bone Gla protein are stimulated, while the synthesis of new collagen I is inhibited (38). Osteoid calcification is further enhanced by the $1,25(OH)_2$VitD-mediated stimulation of Ca and phosphate (P) transport through the osteoid toward the site of mineralization. Additionally, bone formation is stimulated indirectly by the $1,25(OH)_2$VitD-mediated increased release of IGF-I and transforming growth factor-β (TGF-β) by the osteoblasts and chondrocytes, which in their turn enhance bone formation (39). In the process of skeletal remodeling, $24,25(OH)_2$VitD stimulates the maturation of osteoid prior to mineralization (38).

$1,25(OH)_2$VitD has stimulatory effects on different zones of the growth plate, resulting in enhanced chondrocyte proliferation, matrix production, and cartilage mineralization (39).

$24,25(OH)_2$VitD acts predominantly on the resting zone of the growth plate. In vitro, it inhibits chondrocyte proliferation, but stimulates its differentiation and collagen and non-collagen protein synthesis (38). In vivo, the absence of $24,25(OH)_2$VitD during the process of intramembranous ossification, results primarily in an accumulation of non-mineralized osteoid, suggesting that its role in the regulation of this process is more important than in the process of physeal growth and endochondral ossification (38).

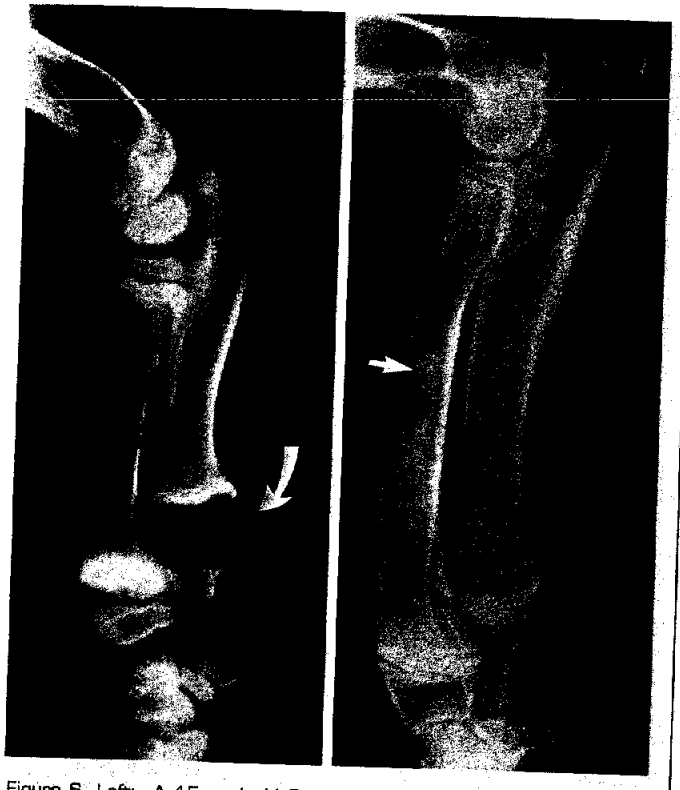


Figure 6. Left: A 15-week-old Great Dane continuously fed a diet with 3.3% calcium (Ca) and 1.1% phosphorus (P) on dry matter base (dmb) from the age of 3 weeks onward, with hypoparathyroidism-induced rickets. Notice the mushroom appearance of the growth plate of the ulna (arrow).
Right: A 21-week-old Great Dane, raised on the same diet with 3.3% Ca and 1.1% P on a dmb between 3 and 6 weeks of age developed severe signs of enostosis characterized by areas of medullary mineralization (34).

The most important role of the calciotropic hormones may, however, be their concerted action to maintain the extracellular Ca concentration - and to a lesser extent the plasma P concentration - within narrow limits, to allow for their deposition on hydroxyappatite within the newly formed bone (38). Other important modulators of physeal growth and endochondral ossification include GH and IGF-I and IGF-II (40).

## DIETARY-INDUCED DISTURBANCES OF ENDOCHONDRAL OSSIFICATION

Longitudinal bone growth is largely dependent on the process of cartilage growth followed by endochondral ossification, as it takes place in the growth plates and in epiphyseal cartilage. Concomitantly, periosteal bone apposition and bone modeling take place. The coordination of the complex and synchronously occurring processes of proliferation, enlargement, and apoptosis of chondrocytes, calcification of the extracellular matrix, and capillary invasion of the mineralized cartilage involve many factors, which act in autocrine, paracrine, and endocrine modes. Among the latter are the calciotropic hormones, acting directly and indirectly via the induction of other regulating factors (36). Disturbance of the normal progress of one of the processes involved in longitudinal bone growth, as can be induced by dietary inadequacies, will lead to an increase in physeal width. Failure of matrix calcification leads to cessation of apoptosis of the chondrocytes, so the growth of capillaries into the lacunae is arrested and thus the formation and subsequent resorption of primary spongiosa is delayed (31, 32).

Nutritional hypovitaminosis D may be induced by dietary VitD deficiency, or an inability to absorb VitD. Plasma concentrations of 25(OH)VitD, 24,25$(OH)_2$VitD and 1,25$(OH)_2$VitD are decreased (18, 19) resulting in decreased Ca and P absorption. This disorder is characterized by disturbance of mineralization not only of the newly formed physeal and epiphyseal cartilage, but also of osteoid (Figure 5). Severe dietary P deficiency also leads to disturbed cartilage and osteoid mineralization. Hypophosphatemia stimulates the 1α-hydroxylation of 25(OH)VitD towards 1,25$(OH)_2$VitD, independent of PTH, and thus promotes Ca and P absorption. Since P is not available for absorption its concentration in the extracellular fluid remains to be too low and thus Ca cannot be sequestered as calcium phosphate in mineralizing tissues. The hypoparathyroidism induced by hypercalcemia suppresses osteoclasia and 1α-hydroxylase, and thus the synthesis of 1,25$(OH)_2$VitD (23). This results in the continuing hypophosphatemia and thus failure of Ca and P precipitation onto cartilage and osteoid, as occurs in hypovitaminosis D (Figure 6, left) (34).

Dietary Ca excess during a short period in the young dog, not causing hypercalcemia, hypoparathyroidism and hypophosphatemia, but only hypercalcitoninism (34) will have consequences for bone remodeling performed by osteoclasts. This can be manifested as enostosis with lameness and intra medullary formation of calcifications (Figure 6, right) (19, 34).

Chronic dietary Ca excess, especially when combined with a proportional increase in P, can cause osteochondrosis in young growing dogs of large breeds with severe clinical consequences (16, 34), but with only mild consequences in more mature puppies (16). High Ca intake in dogs of small breeds causes only microscopic disturbances in endochondral ossification (30). Olsson (1993) and others consider a failure of chondrocyte maturation, leading to retained matrix maturation and calcification, to be the primary event in the development of osteochondrosis. Cartilage continues to grow but since endochondral ossification does not take place, the growth plate increases in width. The nutrition of epiphyseal cartilage depends on diffusion from the synovial fluid and the nutrition of growth plate cartilage on epiphyseal blood vessels. Particularly in immature joint cartilage, the increased thickness of the cartilage may result in cell death in the deeper layers. However, Ekman and Carlson (1998) consider obliteration of cartilage canals to be the primary cause. These canals are found in growing cartilage and may contain both blood and lymph vessels, which are thought to play a role in cartilage nutrition (26), although their function is questioned by others (42). Obliteration of the vessels may cause hypoxia and thus focal necrosis of cartilage as well as hindering ingrowth of vessels from the metaphysis. The cascade of events to complete endochondral ossification fails when the area of necrotic cartilage reaches the ossification front. In osteochondrosis, the defective maturation of the chondrocytes may be focal, leading to irregularity of the metaphysis and epiphyseal cartilage. Micro trauma may lead to fissures in the articular cartilage, creating a cartilage flap within the joint and thus osteochondritis dissecans (31). In growth plates, osteochondrosis may lead to retained cartilage cones and the delay of ossification of the secondary ossification centers (41).

Chronic dietary Ca deficiency causes alimentary secondary hyperparathyroidism. The high plasma PTH concentration stimulates 1,25$(OH)_2$VitD production, and thus intestinal Ca and P absorption, and increased excretion of P with the urine (which is not limited to a maximum) (4). In addition, Ca and

P are released from bone by PTH-induced osteoclasia (Figure 7). This results in near- normal plasma Ca and P concentrations and allows for normal mineralization of cartilage and osteoid (19, 30).

## CONCLUSIONS

Ca plays a vital role in different biological processes. Both dietary Ca and/or VitD deficiencies and dietary Ca excess have consequences for bone mineralization and skeletal development. Chronic excessive Ca intake, with or without a concomitant rise in P intake, has major consequences for both hormonal regulation of Ca metabolism and skeletal development in the dog. The consequences for skeletal development of dietary deficiency or excess of Ca differ with the age and the expected adult size of the pup.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Ahrén B, and Bergenfelz A. Potential clinical use of the EDTA-infusion test. Eur J Clin Chem Clin Biochem 1993; 31: 353-8.
2. Allen LH. Calcium bioavailablility and absorption: a review. Am J Clin Nutr 1982; 35: 783-808.
3. Azria M. The Calcitonins. Physiology and Pharmacology. Karger, Basel, Switzerland, 1989.
4. Broadus AE. Physiological functions of calcium, magnesium and phosphorus and mineral ion balance. In: Favus MJ (ed.). Primer on the metabolic bone diseases and disorders of mineral metabolism. Raven Press, New York, USA, 1993: pp 41-5.
5. Brown AJ, Zhong M, Finch J, Ritter C, McCracken R, Morrissey J, and Slatopolsky E. Rat calcium-sensing receptor is regulated by vitamin D but not by calcium. Am J Physiol 1996; 270: F454-60.
6. Brown EM. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and the extracellular calcium concentration in normal and abnormal parathyroid tissue. J Clin Endocrinol Metab 1983; 56: 572-81.
7. Brown EM. Extacellular $Ca^{2+}$ sensing, regulation of parathyroid cell function, and role of $Ca^{2+}$ and other ions as extracellular (first) messagers. Physiological Reviews 1991; 71: 371-411.
8. Brown EM. The biology of the extracellular $Ca^{2+}$ -sensing receptor. In: Bilezikian JP, Raisz LG, and Rodan GA (eds.) Principles of bone biology. Academic Press, New York, USA. 1996: pp 243-62.
9. Charles P. Calcium absorption and calcium bioavailability. J Int Med 1992; 231: 161-8.
10. Cloutier M, Brossard J, Gascon-Barré, and D'Amour P. Lack of involution of hyperplastic parathyroid glands in dogs: adaption via a decrease in the calcium stimulation set point and a change in secretion profile. J Bone Miner Res 1994; 9: 621-9.
11. D'Amour P, Labelle F, Lecavalier L, Plourde V, and Harvey D. Influence of serum Ca concentration on circulating molecular forms of PTH in three species. Am J Physiol 1986; 251: E680-7.
12. Dempster DW. Bone remodeling In: Coe FL, and Favus MJ (eds). Disorders of bone and mineral metabolism. Raven Press, New York, USA 1992; pp 355-82.
13. Dobenecker B, Kienzle E,and Matis U. Mal- and overnutrition in puppies with or without clinical disorders of skeletal development. The Munich ESVCN Conference Abstract Book, Munich, Germany, 1997: pp 25.
14. Ekman S, and Carlson CS. The pathophysiology of osteochondrosis. Vet Clin North Am Small Anim Pract 1998; 28: 17-32.
15. Goedegebuure SA, and Hazewinkel HAW. Morphological findings in young dogs chronically fed a diet containing excess calcium. Vet Pathol 1986; 23: 594-605.
16. Hazewinkel HAW, Goedegebuure SA, Poulos PW, and Wolvekamp WThC. Influences of chronic calcium excess on the skeletal development of growing Great Danes. J Am An Hosp Assoc 1985; 21: 377-91.
17. Hazewinkel HAW, Hackeng WHL, Bosch R, Goedegebuure SA, Voorhout G, Brom WE van der, and Bevers MM. Influence of different calcium intakes on calciotropic hormones and skeletal development in young growing dogs. Front Horm Res 1987; 17: 221-32.
18. Hazewinkel HAW, How KL, Bosch R, Goedegebuure SA, and Voorhout G. Ungenügende Photosynthese von Vitamin D bei Hunden. In: Meijer H, and Kienzle E (eds.). Ernährung, Fehlernährung und Diätetik bei Hund und Katze. Tierärztliche Hochschule München, Germany, 1988: pp 125-9.
19. Hazewinkel HAW. Calciotropic hormones and bone metabolism. In: Rijnberk A (ed.). Clinical endocrinology of dogs and cats. Kluwer Academic Publishers, Dordrecht, the Netherlands, 1996: pp 177-95.
20. Heany RP. Calcium. In: Bilezikian JP, Raisz LG, and Rodan GA (eds). Principles of bone biology. Academic Press, New York, USA, 1996: pp 1007-19.
21. Hedhammar A, Wu FM, Krook L, Schryver HF, de LaHunta A, Wahlen JP, Kallfelz FA, Nunez EA, Hintz F, Sheffy BE, and Ryan GD. Overnutrition and skeletal disease. An experimental study in growing Great Dane dogs. Cornell Vet 1984; 64 (Suppl. 5): 1-160.
22. How K, Hazewinkel HAW, and Mol JA. Dietary vitamin D dependence of cat and dog due to inadequate cutaneous synthesis of vitamin D. Gen Comp Endocrinol 1996: 12-8.
23. Hurwitz S. Homeostatic control of plasma calcium concentration. Critical Reviews in Biochemistry and Molecular Biology 1996; 31: 41-100.

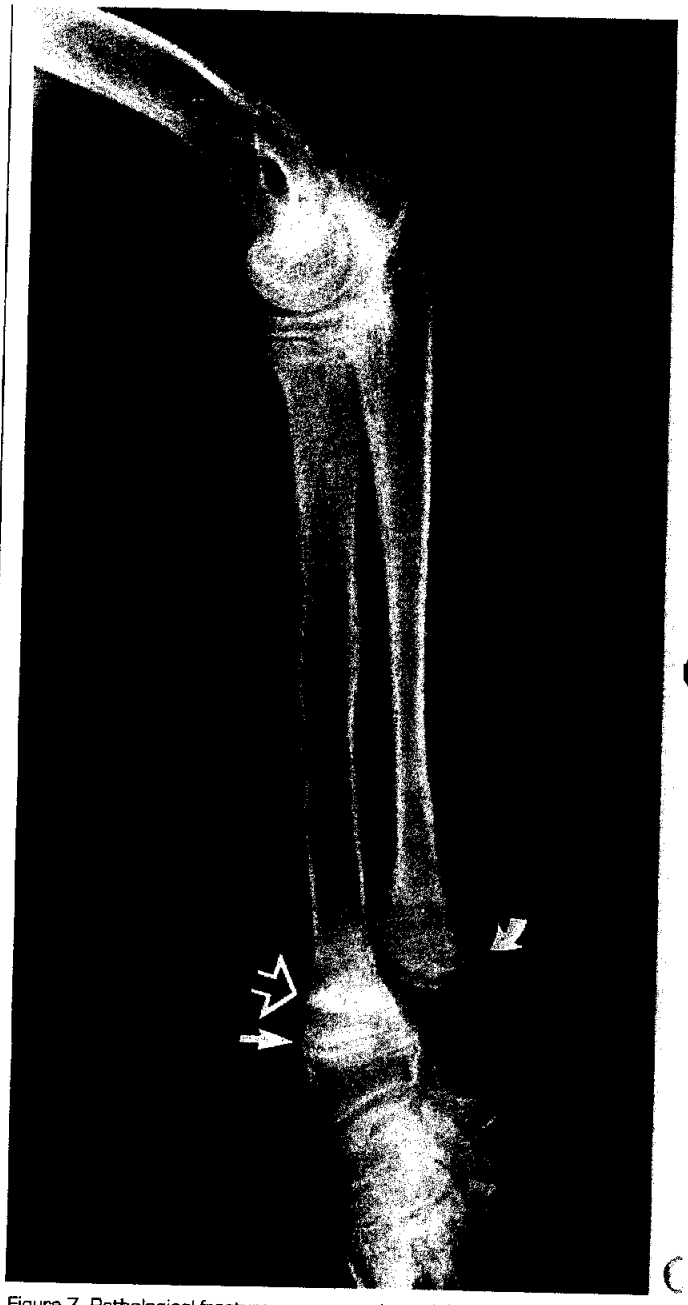

Figure 7. Pathological fractures were seen in a miniature poodle raised on food with a calcium content of 0.05% on dry matter base, here characterized by compression fracture in the radius (open arrow), visible on the radiograph at the age of 4 months (28). Notice the normal alignment of the growth plates of the radius and ulna (solid arrows).

24. Lauten SD, Goodman SA, Brawner WR, Cox NR, Hathcock JT, Jungst SB, Kincaid SA, Montgomery RA, Rumph JT, Baker HJ, Lepine AJ, and Reinhart GA, Growth and body composition of the large breed puppy as affected by diet. In: Reinhart GA, and Carey DP (eds.). Recent advances in canine and feline nutrition. 2nd ed. Orange Frazer Press, Ohio, USA, 1998: pp 63-70.
25. Lemann J jr. Urinary excretion of calcium, magnesium and phosphorus In: Favus MJ (ed.). Primer on the metabolic bone diseases and disorders of mineral metabolism. Raven Press, New York, USA, 1993: pp 41-5.
26. Lufti AM. Mode of growth, fate and functions of cartilage canals J Anat 1970; 106: 35.
27. Mol JA, Kwant MM, Arnold ICJ, and Hazewinkel HAW. Elucidation of the sequence of canine (pro)-calcitonin. A molecular biological and protein chemical approach. Regulatory Peptides 1991; 35: 189-95.
28. Nap RC, Hazewinkel HAW, and Brom WE van den. $^{45}Ca$ kinetics in growing miniature poodles challenged by four different dietary levels of calcium. J Nutr 1993; 123: 1826-33.
29. Nap RC, Hazewinkel HAW, Voorhout G, Brom WE van den, Goedegebuure SA, and Klooster ATh van 't. Growth and skeletal development in Great Dane pups fed different levels of protein intake. J Nutr 1991: S107-S13.
30. Nap RC. Nutritional influences on growth and skeletal development in the dog, Thesis Utrecht University, 1993 [ISBN 90-393-0256-1].
31. Olsson S. Pathophysiology, morphology, and clinical signs of osteochondrosis in the dog. In: Bojrab MJ (ed.). Disease mechanisms in small animal surgery 2nd edition, Lea and Febiger, Philadelphia, USA, 1993: pp 776-96.
32. Reinholt FP, Hjerpe A, Jansson K, and Engfeldt B. Sterological studies on the epiphyseal growth plate in low phosphate, vitamin D-deficiency rickets with special reference to the distribution of matrix vesicles. Calcif Tis Int 1984; 36: 94-101.
33. Richardson DC, and Zentek J. Nutrition and osteochondrosis. Vet Clin North Am Small Anim Pract 1998; 28: 115-35.
34. Schoenmakers I. Modulation of calcium regulation by excessive calcium intake in dogs, studies during different stages of development, Thesis Utrecht University, 1998 [ISBN 90-393-1829-8].
35. Segre GV. Receptors for parathyroid hormone and parathyroid hormone-related protein. In: Bilezikian JP, Raisz LG, and Rodan GA (eds.). Principles of bone biology. Academic Press, New York, USA, 1996: pp 377-403.
36. Silver J, and Naveh-Many T. Vitamin D and the parathyroid gland. In: Feldman D, Glorieux FH, and Pike JW (eds.). Vitamin D. Academic Press, San Diego, USA, 1997: pp 33-40.
37. Slater MR, Scarlett JM, Donoghue S, Kaderly RE, Bonnett BN, Cockshutt J, and Erb HN. Diet and excersice as potential risk factors for osteochondritis dissecans in dogs. Am J Vet Res 1992; 53: 2119-24.
38. St-Arnaud R, and Glorieux FH. Vitamin D and bone development. In: Feldman D, Glorieux FH, and Pike JW (eds.). Vitamin D. Academic Press, San Diego, USA, 1997: pp 293-303.
39. Stern PH. 1,25-Dihydroxyvitamin $D_3$ interactions with local factors in bone remodeling. In: Feldman D, Glorieux FH, and Pike JW (eds.) Vitamin D. Academic Press, San Diego, USA, 1997: pp 341-52.
40. Trippel SB. Growth plate regulation: the somatomedins. Pathol and Immunopathol Res 1988; 7: 43-7.
41. Voorhout G, and Hazewinkel HAW. A radiographic study on the development of the antebrachium in Great Dane pups on different calcium intakes. Veterinary Radiology 1987; 28: 152-7.
42. Wilsman NJ, and Sickle DC van. Cartilage canals, their morphology and distribution. Anat Rec 1972; 173: 9.