Evaluation of Parathyroid Hormone and Preoperative Vitamin D as Predictive Factors for Post-operative Hypocalcemia in Dogs with Primary Hyperparathyroidism.

Master’s Thesis

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Abstract

Primary hyperparathyroidism (PHPTH) results in excessive secretion of parathyroid hormone (PTH) causing hypercalcemia. Vitamin D also contributes to calcium regulation. People with PHPTH and vitamin D deficiency have more severe disease as seen with higher preoperative calcium and longer duration of postoperative hypocalcemia. In people and dogs, a decrease of more than 50% of intraoperative PTH concentration 10 minutes after parathyroidectomy is associated with complete surgical resection of the autonomously functioning gland. Post-operative hypocalcemia after parathyroidectomy necessitates immediate medical intervention and prolonged hospitalization. Distinct pre-operative predictors of postoperative hypocalcemia have not been identified in veterinary medicine.

The objective of this study was to prospectively monitor the trends in PTH concentrations and vitamin D metabolites and determine predictive factors for the development of postoperative hypocalcemia. Our hypothesis was that dogs with a greater than 75% percent change between baseline and intraoperative PTH concentrations will be more likely to develop hypocalcemia in the immediate post-operative period. A second hypothesis is that dogs with low vitamin D status will be more likely to develop postoperative hypocalcemia.
Dogs that developed postoperative hypocalcemia had a significantly larger decrease in PTH concentration than dogs that remained normocalcemic (p<0.008). All dogs had low preoperative calcidiol concentration. This is the first prospective study examining vitamin D and preoperative factors in dogs with PHPTH as predictors of postoperative hypocalcemia.
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Chapter 1: Calcium and Vitamin D

1.1 – Introduction

The regulation of calcium is intricate and complex. The majority of calcium is stored as hydroxyapatite within the skeleton. In the extracellular space, calcium circulates in one of three fractions at approximately; 56% ionized, 34% protein-bound and 10% complexed, with only ionized calcium exerting biological activity\(^1\). Intracellular calcium is maintained in low concentrations, which allows rapid diffusion of ionized calcium from the extracellular fluid into the cytoplasm through calcium sensitive channels. The hormones involved in calcium homeostasis are parathyroid hormone (PTH), produced from the parathyroid glands; calcitriol, the active metabolite of vitamin D; calcitonin from the thyroid gland and calcium itself contributes to the feedback mechanism.

Based on the elimination half-life, PTH is responsible for minute-to-minute adjustment of serum calcium while calcitriol maintains day-to-day control. The concentration of calcium also contributes to calcium homeostasis. Calcium sensing receptors (CSR) located in the parathyroid chief cells, C cells and renal epithelial cells directly regulate intracellular ionized calcium concentration\(^2\). When low serum calcium is detected, PTH secretion is stimulated while elevated serum calcium levels inhibit PTH secretion, however, complete inhibition of PTH secretion does not
occur³. Calcitriol also has an intimate role in calcium homeostasis as it is both a product of PTH stimulation and regulates PTH production. Activation of the renal calcium sensing receptors during times of hypercalcemia causes increased calcium excretion through urine whereas PTH increases tubular reabsorption of calcium⁴.

1.2 - Parathyroid Hormone

Parathyroid hormone (PTH), also known as parathormone or parathyrin, is the only calcium-regulating hormone secreted by the chief cells of the parathyroid glands. Release of PTH is in response to low systemic ionized calcium concentrations. Hypocalcemia stimulates an increased rate of secretion while hypercalcemia and calcitriol inhibits PTH synthesis and secretion⁵. Once released, PTH has a half-life of 3 to 5 minutes due to rapid cleavage by liver Kupffer cells⁶. For this reason a steady rate of secretion occurs to maintain calcium homeostasis. The inverse relationship between PTH and calcium ensure that a small change in calcium will cause a relatively large change in PTH. Disease states such as primary hyperparathyroidism increases the PTH-calcium set point to the right and thus increases the threshold of ionized calcium required to achieve half the maximal PTH secretion⁷,⁸.

PTH acts directly on bone and the kidneys through the parathyroid hormone 1 receptor to increase circulating calcium concentrations and also has an effect on the central nervous system and pancreas via the parathyroid hormone 2 receptor in people⁹. Regulation of PTH is complex with feedback systems involving calcium concentration, calcitonin and calcitriol.
1.2.1 - Molecular structure

PTH is an 84 amino acid polypeptide chain that was first sequenced from the bovine species in 1970\textsuperscript{10, 11}. Subsequently, the porcine PTH molecule and human PTH form was sequenced shortly afterwards\textsuperscript{12}. The hormone molecule is relatively well preserved across species, with few amino acid substitutions. The human PTH molecule contains asparagine at position 16 compared to the serine in the bovine and porcine molecules\textsuperscript{13}. Although this substitution is unique to the human molecular structure, similarities are documented between the human and porcine PTH molecule that differ from the bovine molecule\textsuperscript{13}. The canine PTH molecule was successfully sequenced in the mid 1990’s and was found to be 88% homologous to the human molecule\textsuperscript{14}. There were two substitutions in the (1-40) amino acid sequence at positions 7 and 16 that also occurs in bovine and porcine PTH molecules\textsuperscript{3, 14}.

These early extraction and purification studies identified PTH as the sole hormone synthesized, stored and secreted from the parathyroid glands. It was also determined that there was limited storage capacity for PTH, with estimates of approximately 0.004% PTH per parathyroid gland weight, indicating a high turnover rate for biosynthesis and secretion\textsuperscript{15}. Further research has established that a prohormone molecule is synthesized first by the parathyroid glands and then cleaved into the active PTH molecule. The prohormone has an additional six amino acid sequence, Lys-Ser-Val-Lys-Lys-Arg, at the amino terminus which are subsequently cleaved to form the active 84 amino acid polypeptide for storage and secretion\textsuperscript{16}. The entire 84 amino acid sequence is not
necessary for function as only the (1-34) amino acid sequence, is sufficient for biologic activity\textsuperscript{13}.

1.2.2 - Secretion rhythm

Numerous endogenous hormones are secreted in an episodic, cyclic or rhythmic characteristic rather than continuously, in healthy individuals. Intermittent secretion is known to exist for numerous hormones such as luteotropic and follicle-stimulating hormones, cortisol, aldosterone, insulin and growth hormones\textsuperscript{17-21}. The biologic rhythm of PTH secretion is multifactorial with influences from both endogenous and exogenous variables.

Circadian rhythms are known to influence hormone plasma concentrations due to cyclical activity related to day-night, sleep cycle and activity-inactivity. Diurnal variations in PTH secretion have been demonstrated in humans\textsuperscript{22} and in dogs\textsuperscript{23}. Studies in people reported one maximum PTH peak in the early morning hours leading to a nadir at approximately 10:00 military time\textsuperscript{22, 24, 25}. Due to the nocturnal PTH surge, sleep cycle has also been implicated to affect PTH levels, in particular, PTH release was related to sleep stages 3 and 4\textsuperscript{26}. However, other studies did not support this conclusion and did not show a significant difference in nocturnal rise of PTH when healthy men were subjected to forced wakefulness in constant environmental conditions\textsuperscript{24} and also if the timing of sleep was shifted\textsuperscript{25} (25). Interestingly, calcium concentration also follows a diurnal rhythm\textsuperscript{23, 27}.
Under constant environmental factors with enforced wakefulness and set feeding rations in men, the circadian rhythm of PTH remained relatively preserved, indicating a large endogenous component of PTH secretion that is not affected by diet, posture or sleep-wake cycles\(^{24}\). Other studies have documented a bimodal PTH pattern with a similar primary peak in early morning and a secondary PTH peak in the afternoon\(^{28}\).

There is also a significant seasonal variation of PTH secretion in people, with a decline of 20% below the annual mean during summer seasons and 20% increase during winter seasons\(^ {29}\).

1.2.3 – Regulation

The rate of PTH secretion is inversely proportional to the concentration of serum calcium\(^ {30}\). The major stimulus of PTH secretion is hypocalcemia, which triggers rapid PTH secretion from stored reserves and concurrently inhibits intracellular degradation of PTH. A rapid decrease in circulating iCa by 2-3% can trigger a 400% increase in PTH secretion\(^ {31}\). In states of prolonged hypocalcemia, up regulation of PTH gene expression and the proliferation of chief cells occur to maximize PTH secretion. Measures used to protect against hypercalcemia include up regulation of intracellular PTH degradation within the parathyroid glands and inhibition of PTH gene transcription when calcitriol activates the vitamin D receptor (VDR)\(^ {3}\). Negative feedback by the arachidonic acid pathway also contributes to rapidly decreasing PTH secretion\(^ {4}\).
1.3 - PTH Target organs

PTH exerts direct effects on the skeleton and kidney and has indirect effects on the intestines. The effect of PTH on the skeleton is biphasic with an immediate “rapid” phase and a delayed “slow” phase. In the rapid phase called “osteolysis,” PTH is bound to osteoblasts and osteocytes and results in increased calcium-phosphate salt absorption. The slow phase of PTH’s effect on bone is caused by osteoblastic activation of osteoclasts resulting in prolonged bone resorption and bone remodeling. PTH increases osteoclast formation to increase bone resorption and the release of calcium. In the kidneys, PTH causes reabsorption of calcium in the distal tubules, collecting tubules and to a lesser extent from the ascending loop of Henle. PTH also causes rapid loss of phosphorus in the urine by decreasing reabsorption in the proximal tubules. PTH activates and induces synthesis of the enzyme 1α-hydroxylase in the renal epithelium of the proximal convoluted tubules, which is responsible for the conversion of calcidiol (25-hydroxycholecalciferol) to the active vitamin D metabolite 1,25-dihydroxycholecalciferol (calcitriol). Calcitriol contributes to elevating circulating calcium concentration by acting on enterocytes to increases intestinal absorption of calcium and phosphorus.

1.4 - Vitamin D

Vitamin D is a fat-soluble prohormone. There are two main forms of Vitamin D, ergocalciferol (vitamin D2), which is of plant origin or cholecalciferol (vitamin D3) that can be synthesized by skin or acquired from other animal tissue. In mammals, Vitamin D can be obtained from dietary intake or by dermal synthesis from sunlight. The skin of
humans, horses, pigs, rats, cattle and sheep contain adequate quantities of 7-dehydrocholesterol which can be converted to cholecalciferol, however the skin of cats and dogs do not contain a significant quantity of 7-dehydrocholesterol making dietary intake in these species essential.\textsuperscript{32}

Calcitriol, 1,25-dihydroxyvitamin D (1,25(OH)_2D), is the most biologically active form of vitamin D and is essential for maintenance of calcium and phosphate homeostasis, normal bone formation, cellular growth control and differentiation of various tissues.\textsuperscript{33, 34} Cholecalciferol requires two enzyme conversions to become the active molecule, calcitriol. In the liver, cholecalciferol is converted to calcidiol, 25-hydroxyvitamin D (25(OH)D), by 25-hydroxylase and then by 1α-hydroxylase to 1,25(OH)_2D in the kidney.

The major site of vitamin D metabolite regulation is through the activity of renal 1α-hydroxylase, which is up regulated by PTH.\textsuperscript{35, 36} Extrarenal sites of 1α-hydroxylase have also been reported in keratinocytes, testicles, brain, cultured bone cells, macrophages, placenta, prostate cells, islet cells of the pancreas and the parathyroid glands.\textsuperscript{37-44} Extrarenal 1α-hydroxylase results in local production of 1,25(OH)_2D for autocrine or paracrine regulation compared to the endocrine function of renal 1α-hydroxylase.\textsuperscript{45}

Calcitriol circulates in the blood and binds to the vitamin D receptor (VDR) to activate intracellular transcription factors and modulate gene expression.\textsuperscript{33} Regulation of calcium homeostasis is one of the major functions of 1,25(OH)_2D mainly targeting the intestines, kidneys, bone and the parathyroid glands.
1.5 - Impact of Vitamin D and calcium regulation

It is well known that the role of vitamin D has significant effect on calcium regulation. Vitamin D and PTH secretion have an inverse relationship where decreased vitamin D causes an increased synthesis of PTH. Geographic and seasonal associations in plasma vitamin D concentrations exist in people with higher 25-hydroxyvitamin D levels in late summer seasons.

1.5.1 - Role of vitamin D on the intestines

Calcitriol is the main factor in regulating intestinal calcium absorption secondary to dietary intake. Binding of 1,25(OH)₂D to VDR activates transport of calcium either by passive diffusion mechanism via paracellular pathway or actively through a transcellular pathway. The paracellular pathway predominates when dietary levels of calcium are high with calcium transport through tight junctions. The transcellular pathway involves transport of calcium from the brush border in the duodenum and jejunum through the transient receptor potential vanillinoid 6 (TPRV6), followed by cystolic binding to the calcium binding protein calbindin and then excretion through the plasma membrane calcium ATPase on the basolateral membrane. This pathway predominates when dietary calcium levels are normal to low.
1.5.2 - Role of vitamin D on the kidneys

Regulation of calcium reabsorption in the kidneys occurs in the distal nephron. Binding of 1,25(OH)₂D to renal VDR stimulates an active calcium transcellular pathway whereas the proximal tubule is responsible for passive resorption of a large part of the filtered calcium\(^{50}\). The active transcellular resorption of calcium occurs in the distal tubule into the cytoplasm through the TPRV-5, followed by coupling to calcium binding proteins and then excretion through the plasma membrane calcium ATPase and sodium-calcium exchanger on the basolateral membrane\(^{49}\). TRPV5 is the rate-limiting step of renal calcium resorption is regulated by binding of the fibroblast growth factor-23 and Klotho complex\(^{49,51}\). Experimental Vdr null mice have significant calciuria despite systemic hypocalcemia due to impaired renal calcium reabsorption\(^{52}\). The amount of renal calcium reabsorption varies depending with the amount of intestinal calcium absorption.

1.5.3 - Role of vitamin D on bone

One of the main roles of the vitamin D system is to maintain calcium homeostasis even at the expense of bone mass and strength. The effect of calcitriol on bone is dependent on the overall calcium balance such that during positive calcium balance, calcitriol regulates bone metabolism\(^{53}\). The skeleton serves as a calcium reserve when insufficient calcium is obtained from the intestines and kidneys. VDR null mice that are calcium deficient develop osteopenia and spontaneous fractures\(^{53}\). During negative
calcium balance, calcitriol will prioritize maintaining normocalcemia by increasing bone resorption and suppression of bone mineralization\textsuperscript{53}.

1.5.4 - \textit{Role of vitamin D on parathyroid glands}

Calcitriol directly plays an important role in the feedback regulation of parathyroid function by causing suppression of PTH transcription and secretion. Calcitriol is also known to inhibit parathyroid cell proliferation\textsuperscript{43}. By decreasing PTH secretion, calcitriol indirectly regulates the synthesis of 1,25(OH)\textsubscript{2}D by limiting the activity of 1\textalpha\-hydroxylase, a PTH-induced enzyme, activity in the kidney\textsuperscript{54}. An overexpression of 1\textalpha\-hydroxylase was identified in parathyroid adenomas and in hyperplastic parathyroid glands associated with secondary hyperparathyroidism compared to normal parathyroid glands. It is believed that this increased expression of 1\textalpha\-hydroxylase may lead to increased local production of 1,25(OH)\textsubscript{2}D in an effort to control parathyroid cell proliferation\textsuperscript{43}. Although the formation of 1,25(OH)\textsubscript{2}D may be based on availability of circulating 25(OH)D, it was found that when 25(OH)D was administered, individuals with PHPTH had a significantly higher circulating 1,25(OH)\textsubscript{2}D compared to normal controls\textsuperscript{55}. People with chronic vitamin D-deficiency may have accelerated parathyroid adenoma growth and aggravate skeletal changes\textsuperscript{56, 57}. 
Chapter 2: Primary Hyperparathyroidism in Dogs

Hyperparathyroidism is a process associated with the excessive secretion of parathyroid hormone (PTH) in circulating blood. Primary hyperparathyroidism (PHPTH) indicates hyperfunctioning parathyroid glands independent of circulating ionized calcium concentration; secondary hyperparathyroidism occurs in response to prolonged states of hypocalcemia as seen with chronic kidney failure or with vitamin D deficiencies. Tertiary hyperparathyroidism is a condition reflecting the development of autonomous parathyroid function following a period of secondary hyperparathyroidism resulting in both elevated PTH and presence of ionized hypercalcemia.\(^{58}\)

In health, elevated plasma circulating calcium levels inhibit PTH secretion while low serum calcium levels stimulate PTH release from non-pathologic parathyroid glands. Even in the presence of severe hypercalcemia, complete inhibition of PTH secretion does not occur.\(^{3,14}\) People with PHPTH have altered physiologic calcium homeostasis that increases the calcium set point and requires a greater concentration of calcium to inhibit PTH secretion.\(^{59}\)

PHPTH is usually clinically diagnosed following the discovery of increased circulating ionized calcium. PTH is secreted by parathyroid chief cells and inversely fluctuates with circulating ionized calcium concentrations. A diurnal peak of PTH
secretion has been reported in dogs, in which the morning PTH concentration was approximately twice the lowest concentration during the day. The short half-life of circulating PTH necessitates a steady rate of secretion to maintain calcium homeostasis. The concept of normocalcemic primary hyperparathyroidism has been described in people and is a disorder of mineral metabolism and excessive PTH secretion with normal calcium levels. As PTH and calcium have cyclic secretion patterns, diagnosis of normocalcemic primary hyperparathyroidism require consistently normal albumin-adjusted total serum calcium and normal ionized calcium with elevated PTH. In addition, secondary causes of elevated PTH such as vitamin D deficiency, hypercalcuria, concurrent gastrointestinal malabsorption conditions and PTH inducing medication should also be ruled out.

2.1 - PHPTH in dogs

PHPTH was first recognized in veterinary medicine in 1958 and has historically been reported as uncommon in dogs and rare in cats. Literature review over the past two decades yielded few large case series with an abundance of smaller case series and case reports. Recent literature has suggested the incidence of PHPTH is more common than historically reported.

Approximately 90% of dogs and cats have been reported with one solitary hyperfunctioning gland. In another study, 42% of dogs were found to have two abnormal parathyroid glands. The mean age of diagnosis is approximately 11 years old and ranges from adult to geriatric (6-17 years old) dogs with no sex predilection. An
autosomal dominant genetic inheritance pattern predisposes Keeshond dogs to PHPT with odds ratio of 50.7 (75). Siamese cats may be at increased risk 76.

2.2 - Anatomy of the parathyroid glands

In dogs, the parathyroid glands are ellipsoid discs measuring 2 to 5 mm in diameter and 0.5 to 1 mm in width 77. The four most regularly occurring parathyroid glands are the external and internal parathyroid glands, also named parathyroid III and IV respectively referring to the embryologic pharyngeal pouch 78. The external parathyroid gland is usually located at the cranial dorsolateral margin of the thyroid gland and the internal parathyroid gland tends to be embedded within the caudal portion of the thyroid gland. Variations in location to both the external and internal parathyroid gland have been described 78, 79.

Accessory or ectopic parathyroid glands are rare with a reported incidence of 3-6% in dogs and 35-50% in cats 80, 81. Locations of ectopic parathyroid gland vary, but can be visualized on serial sections of the thyroid gland 82 or may migrate into the thorax with the thymus 78. Earlier publications have identified as many as 13 parathyroid glands in one dog 61, 83. Ectopic glandular tissues from the cervical to thoracic inlet has been described and are related to the embryonic development from the third and fourth pharyngeal pouch 61.
2.2.1 - *Vascular supply*

In the dog, blood supply, lymphatic drainage and innervation of the parathyroid glands are related to the thyroid gland. The main blood supply is via the cranial and caudal thyroid arteries. The cranial thyroid artery is the first major branch off the common carotid artery and supplies the cranial pole of the thyroid gland. One smaller vessel from the cranial thyroid artery supplies the external parathyroid gland. The caudal thyroid artery arises from a branch of the brachiocephalic artery to supply the caudal pole of the thyroid gland and anastomoses with the cranial thyroid artery. The internal parathyroid gland is supplied by blood vessels that surround the thyroid parenchyma. Venous drainage parallels arterial supply with the cranial and caudal thyroid vein draining into the internal jugular vein. Lymphatic drainage of the parathyroid gland is to the retropharyngeal lymph nodes. This is one of the few glands that have lymph drainage cranial to the organ\(^7\).

2.2.2 - *Nervous system*

The thyroid nerve is formed from fibers from the cranial cervical ganglion and cranial laryngeal nerve. The principle innervation to the parathyroid glands is derived from the autonomic nervous system. The specific role of the autonomic nervous system in relation to glandular secretion is not clearly understood, but is believed to have effects on blood perfusion rates to the gland\(^7\).
2.3 - Impact of vitamin D deficiency and PHPTH

People with PHPTH with concurrent vitamin D deficiency are associated with more severe disease as seen with higher calcium, higher alkaline phosphatase, higher baseline PTH and larger parathyroid glands\(^43, 46, 57, 84\).

The definitions of vitamin D deficiency and insufficiency are highly controversial in people with vitamin D deficiency as defined as 25-hydroxyvitamin D <20 ng/ml whereas vitamin D insufficiency is 20-29 ng/ml and vitamin D sufficient identified as >30 ng/ml in a population of PHPTH people\(^46\). Others suggest vitamin D insufficiency between 10-20 ng/ml (25-50 nmol/L) and vitamin D deficiency below 10 ng/ml (25 nmol/L). Vitamin D levels between 4.8-10 ng/ml (12-25 nmol/L) will cause proximal myopathy or increased bone turnover and levels below 4-4.8 ng/ml (10-12 nmol/L) were associated with frank osteomalacia\(^85, 86\).

2.4 - Clinical signs of PHPTH

Clinical signs in dogs with PHPTH tend to be mild and include polydipsia, polyuria, weakness, lethargy, gastrointestinal disease, stranguria, pollakuria, urethral obstruction\(^87, 88\). Approximately 40% of dogs may be asymptomatic\(^62\). Polyuria and polydipsia are often the most common presenting compliant\(^62\). Of 210 dogs with PHPTH, 31% of dogs had urolithiasis and 29% of dogs had urinary tract infections\(^62\). Gastrointestinal signs are secondary to reduced excitability of gastrointestinal smooth muscle\(^4\). To compensate for dehydration from obligatory polyuria, polydipsia ensures. Due to impaired renal concentrating abilities, dogs are prone to urinary tract infections
with or without urinary stones. Calcium oxalate stones form secondary to hypercalciuria and can cause urethral obstruction\textsuperscript{4, 87}.

\textbf{2.5 - Diagnostics}

Investigation into a diagnosis of PHPTH is usually initiated after an elevated total serum calcium level is reported on blood work. Dogs with PHPTH, especially compared to dogs with other causes of hypercalcemia are generally healthy compared to other dogs with hypercalcemia\textsuperscript{87}.

\textbf{2.5.1 - Blood work}

In veterinary medicine, elevated total calcium concentration is the hallmark abnormality detected prompting further investigation. Serum calcium exists in three forms: 56\% ionized (biologically active form), 10\% complexed (bound to phosphate, bicarbonate, sulfate, citrate and lactate) and 34\% protein-bound\textsuperscript{4}. Elevation in total calcium may not reflect an elevation in the ionized fraction of calcium. Although changes in albumin, globulin, protein content, presence of lipemia or hemoconcentration of plasma or serum can alter total calcium results, the concentration of ionized calcium is not usually affected\textsuperscript{87}. Presence of acidemia decreases calcium binding to protein and consequently increases the ionized calcium. Ionized calcium may be a more sensitivity indicator of systemic calcium than total calcium. Reports in people have identified that although patients with elevated total calcium also had elevated ionized calcium, a subset of patients with normal total calcium had elevated ionized calcium levels\textsuperscript{89}. This
diagnostic discordance has also been described in dogs\textsuperscript{31}.

Serum phosphorus concentration in dogs with PHPTH is often reported to be below the reference range or within the lower half of the reference range. This occurs following PTH-induced renal inhibition of proximal tubular phosphorus resorption\textsuperscript{4}. Approximately 65\% of dogs had low serum phosphorus concentration and 34\% had serum phosphorus concentration within reference ranges\textsuperscript{62}. The presence of hypophosphotemia or normophosphotemia rules out primary renal disease and secondary hyperparathyroidism as causes for hypercalcemia. Alkaline phosphatase (ALP) may be elevated in dogs with PHPTH due to increased bone turnover from osteoclastic activity\textsuperscript{63}. Utilizing duel-energy X-ray absorptiometry (DEXA) technology can quantify bone density. These studies have not been performed in dogs with PHPTH. Increased ALP is correlated with development of rapid and prolonged postoperative hypocalcemia and hungry bone syndrome in people\textsuperscript{90}.

Hemogram changes are minimal and associated with dehydration from polyuria and polydipsia\textsuperscript{87}. Common urinalysis findings include isosthenuria or hyposthenuria, hematuria, pyuria and crystalluria secondary to polyuria and polydipsia, urinary tract infections and/or uroliths\textsuperscript{87}.

2.5.2 – \textit{PTH analysis}

Diagnosis of PHPTH is based on normal or elevated serum PTH concentrations in the face of ionized hypercalcemia. This is an inappropriate response since high circulating ionized calcium levels should inhibit PTH synthesis and release\textsuperscript{87}. In one
report, approximately 75% of dogs with PHPTH had a PTH concentration within the reference range\textsuperscript{62}.

PTH is an 84 amino acid polypeptide chain that can be analyzed by immunoradiometric assay (RIA) or by chemiluminescent assay (CLA). The first 34 amino acid sequences, which is approximately 30-40\% of the entire sequence, is sufficient for biologic activity\textsuperscript{13}. Many challenges with the accuracy of RIA have been identified over the years. Early assays that rely on detection of the mid-region sequence or C-terminus of PTH may be falsely elevated in certain disease conditions of delayed renal clearance such as in chronic kidney disease\textsuperscript{91}. Fine-tuning of the first generation RIA have improved the sensitivity of PTH detection and the third generation tests utilizing antibodies directed at the C-terminal region in conjunction with antibodies directed at the first four amino acid of the sequence at the N-terminal to detect the intact PTH molecule\textsuperscript{92}. Intact PTH assays have been reported to overestimate the presence and severity of parathyroid-mediated osseous abnormalities in uremic patients\textsuperscript{93}. In addition, there is discordance between different assay manufactures making comparing PTH values challenging\textsuperscript{92}. Compared to the RIA the CLA is a rapid in-house test that can be performed at many specialty institutions. The CLA can also detect the biologically active intact PTH molecule. Due to the quick turnaround of results, intraoperative PTH testing has revolutionized the standard of care for parathyroidectomy in people\textsuperscript{94}. As PTH is relatively well preserved across species, human PTH assays have been validated for use in dogs\textsuperscript{67, 68}.
Although few larger specialty veterinary hospitals may have access to an in-house CLA analyzer, the majority of general practitioner veterinarians ship serum to the Michigan State University – Diagnostic Center for Population and Animal Health (MSU-DCPAH) laboratory for RIA PTH analysis. The exact methodology of the MSU-DCPAH for PTH analysis is proprietary and not available for review. Even though the PTH molecule is relatively preserved across species, the variations between species and post-translational modifications can alter the affinity of the animal PTH molecule for the anti-PTH antibody used in the assay. Other species-specific molecules or molecules associated with certain pathologic conditions might interfere with PTH detection. At this institution, the older in-house model of the analyzer prohibits use of CLA technology for PTH analysis and samples are shipped to MSU-DCPAH for analysis.

2.5.3 - Intraoperative PTH Assay

Rapid intraoperative PTH assay has high accuracy, approximately 95%\(^9\), associated with surgical excision of all autonomously functioning parathyroid tissue in people.\(^9\) Circulating half-life of PTH in people with normal functioning kidneys ranges from 1.68-21.5 minutes.\(^9\) For intraoperative testing in people, baseline PTH concentration is obtained prior to parathyroidectomy and compared to a blood sample obtained at a set time point after the affected parathyroid gland has been removed.\(^9\) A decrease of more than 50% between post-excision and pre-excision samples was indicative surgical cure.\(^9\)
Since the PTH molecule is relatively conserved between species, a CLA PTH assay has recently been validated in dogs. In that study, all dogs with uniglandular disease had more than 50% decrease in PTH concentration after parathyroidectomy, while dogs with multiglandular disease required removal of both abnormal parathyroid glands prior to the PTH concentration decreasing to more than 50% compared to pre-excision. Intraoperative PTH analysis has also identified a significant increase in PTH concentration was associated with surgical manipulation of the affected gland in dogs.

2.5.4 – Vitamin D analysis

One of the main roles of the vitamin D system is to maintain calcium homeostasis even at the expense of bone mass and strength. The bone serves as a calcium reserve when insufficient calcium is obtained from the intestines and kidneys. Vitamin D plays an integral role in PTH synthesis and calcium regulation and should be evaluated during workup of PHPTH.

Circulating concentrations of 1,25(OH)₂D are tightly regulated by the body while the major circulating form of vitamin D is 25-hydroxyvitamin D which is the best indicator of available vitamin D in humans and animals. Similar to PTH analysis, vitamin D is evaluated by either RIA or CLA. Variations in the recognized antibodies are associated with IRIA methodology with some assays recognizing both 25OHD₂ and 25OHD₃, whereas others underestimate 25OHD₂.
2.6 – Diagnostic imaging

Imaging of parathyroid glands can be challenging due to their small size and superficial location. A gold standard parathyroid imaging modality in people has not been recognized, however cervical ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and nuclear scintigraphy have all been used to localize affected parathyroid glands\textsuperscript{100-106}.

2.6.1 – Ultrasound

Ultrasonographic images of normal parathyroid glands in normocalcemic animals are not commonly seen and when observed are usually less than 2-3 mm in length, round or oval in shape and anechoic or hypoechoic compared to surrounding thyroid parenchyma\textsuperscript{107-109}. The most common structure misinterpreted for parathyroid glands were thyroid lobules, thyroid cysts, parathyroid cysts, lymphoid tissue and vasculature\textsuperscript{100, 109}. Other causes of misidentification include ectopic location of parathyroid tissue, parathyroid tissue deep to the trachea or parathyroid tissue that is isoechoic to the thyroid glands or too small to distinguish\textsuperscript{109}.

Neoplastic lesions, adenoma or adenocarcinoma of the parathyroid glands, measure greater than 4 mm in length while hyperplastic parathyroid glands are between 3-4 mm in size\textsuperscript{107}. Good correlation has been reported comparing ultrasound measurements and gross parathyroid measurements\textsuperscript{107, 109}. The success of ultrasound in the identification of normal an abnormal parathyroid glands is user dependent. Without
cytology or histopathology, it is impossible to differentiate parathyroid nodule from other thyroid pathologies.

2.6.2 - Nuclear scintigraphy

Nuclear scintigraphy is a commonly used imaging modality in people for detection and localization of hyperfunctioning parathyroid tissue. Organs absorb radioisotopes and emit gamma radiation that is captured with scintillation, or gamma cameras. The most common radioisotopes used are thallium, sestamibi and tetrofosmin.\(^{101}\)

Subtraction technique requires two different radioisotopes to detect abnormal parathyroid tissue.\(^{106}\) The thyroid gland absorbs both Thallium \(^{201}\text{TI}\) and pertechnetate-
\(^{99}\text{Tc}\) radioisotopes while hyperfunctioning parathyroid glands only take up \(^{201}\text{TI}\) but not \(^{99m}\text{Tc}\) pertechnetate. Once acquired, the images are subtracted from each other to identify the abnormal parathyroid tissue.\(^{106}\)

Double-phase scintigraphy with a single agent uses Technectium-\(^{99m}\text{Tc}\)-sestamibi (\(^{99m}\text{Tc}\)-sestamibi). Success of this procedure is based on the differential washout of \(^{99m}\text{Tc}\)-sestamibi from thyroid and parathyroid tissue.\(^{102}\) Hyperfunctioning parathyroid glands will absorb the radioisotope in a similar fashion as thyroid tissue during the initial phase, and will remain radioactive through the delayed phase.\(^{102}\) Double phase scintigraphy has a sensitivity of 83-95% for detection of parathyroid adenoma\(^{102-104}\) and a 37-62%\(^{103,104}\) for parathyroid hyperplasia in people. The specificity of parathyroid scintigraphy is difficult to assess since unaffected patients rarely undergo diagnostics. In
one scintigraphy study, a specificity of 75% was reported as three of four patients who
did not have parathyroid disease had negative scans\textsuperscript{103}.

Limited reports of scintigraphy use for diagnosing and localizing abnormal
parathyroid glands are available in veterinary medicine\textsuperscript{110,111}. One case report with
scintigraphy correctly identified the presence and location of a parathyroid adenoma\textsuperscript{110}.
In a larger study, the overall sensitivity of parathyroid scintigraphy was 11%, specificity
of 50% and overall accuracy of 27% with a sensitivity of 25% for localizing parathyroid
adenoma and 0% for parathyroid hyperplasia\textsuperscript{111}.

Possible explanations for this discrepancy between success of parathyroid
scintigraphy in humans and dogs may be related to parathyroid size. Hyperfunctioning
parathyroid glands in dogs measured 0.3-2.0 cm\textsuperscript{111} compared to abnormal human
parathyroid glands ranging from 1.2-6.5 cm in diameter\textsuperscript{105}. In another study, the smallest
adenoma detected by scintigraphy measured 0.7 cm in diameter in people\textsuperscript{112}. It is also
possible that dogs with hyperfunctioning parathyroid glands have a faster washout time
than compared to abnormal human parathyroid glands necessitating different image
capture time. Other limitations of scintigraphy in veterinary medicine involve cost of
equipment, appropriate housing and disposal of waste for radioactive patients and trained
personnel.

\textbf{2.6.3 - Computed Tomography/Magnetic Resonance Imaging}

Advanced imaging with CT and MRI are usually reserved for localization of
suspected ectopic parathyroid tissue after failed initial treatment. Most functional
parathyroid adenomas are hyperenhancing on CT, which distinguishes them from lymph nodes. In humans, MRI images are obtained from the hyoid bone to the sternal notch. Parathyroid tissue has variable intensity on MRI, but typically shows intermediate-to-low signal intensity on T1-weighted images and high signal intensity on T2-weighted images\(^\text{101}\). The sensitivity and specificity of CT/MRI to diagnose and localize functional parathyroid glands in veterinary medicine is not reported. Limitations due to cost of procedure, necessity of general anesthesia and availability of the equipment make CT and MRI less commonly used in veterinary medicine.

2.7 - Pre-treatment

Definitive guidelines of when to initiate pre-treatment prior to parathyroidectomy is lacking in veterinary medicine. Conflicting correlation of preoperative calcium levels and developing postoperative hypocalcemia is reported in people\(^\text{113}\). The current recommendation in people is to pretreat with vitamin D metabolites in individuals with vitamin D deficiency prior to parathyroidectomy\(^\text{114}\).

If the level of hypercalcemia necessitates management prior to ablation procedure or surgery, a variety of treatments are available such as saline diuresis, diuretic therapy, glucocorticoids, bisphosphonates and calcitonin. The majority of treatment is aimed at increasing calciuresis in the kidneys with saline diuresis or addition of furosemide. Glucocorticoids will also reduce bone resorption of calcium and bisphosphonates and calcitonin have inhibitory effects on osteoclastic bone resorption activity\(^\text{87}\). The goal of pretreatment with an active vitamin D metabolite such as calcitriol is to lessen the
severity or prevent the onset of post-operative hypocalcemia, by increasing enterocyte absorption of calcium into the circulation to counteract the acute decrease in PTH production following removal of hyperactive parathyroid tissue. This topic remains controversial to the type of medication used, dose and ideal duration prior to surgery.

Dogs with preoperative serum total calcium concentration less than 14 mg/dl have a small risk of developing postoperative hypocalcemia in the opinion of some authors. Consideration for prophylactic treatment with calcitriol for dogs with serum total calcium greater than 15 mg/dl has been recommended. In one study, dogs that did not experience tetany after parathyroidectomy had preoperative mean total calcium of 13.7 mg/dl compared to dogs that experienced tetany that had mean total calcium of 15.4 mg/dl. In that study, 11 of 12 dogs that developed postoperative hypocalcemia were not prophylactically treated preoperatively with calcium salts or calcitriol. In another study, dogs with mean preoperative total calcium 13.7 mg/dl did not develop hypocalcemia compared to dogs with mean preoperative total calcium of 16.8 mg/dl that developed postoperative hypocalcemia. In one study, 13 of 19 dogs were pretreated with alfacalcidiol, a hydroxylated vitamin D analogue, with or without calcium carbonate prior to surgery. The amount of pretreatment time varied from one day prior to surgery to initiating pretreatment the day of surgery.

The vitamin D metabolite of choice is calcitriol, compared to cholecalciferol and ergocalciferol, due to its short time to achieve biological effect (1-4 days) and short circulating half-life. The proposed dosing regimen and duration for calcitriol is 10-20 ng/kg orally every 12 hours starting 3 to 5 days prior to surgery for 2-3 days, then
decreasing the dose to 5 ng/kg orally every 12 hours until surgery\textsuperscript{115}. This recommendation has been controversial since it is not known whether this short course of calcitriol would be sufficient time to prime the gastrointestinal system to adequately increase calcium absorption.

### 2.8 - Treatment Options

Current mainstay treatment options in veterinary medicine are with ultrasound-guided heat ablation or parathyroidectomy.

#### 2.8.1 - Ethanol ablation

Percutaneous ethanol ablation of the parathyroid glands for PHPTH in dogs was first described in 1999\textsuperscript{40}. Ethanol causes coagulation necrosis and vascular thrombosis of exposed tissue\textsuperscript{87}. The volume of the pathological parathyroid gland is approximated with ultrasonography and a matched volume of 96% ethanol is injected into the pathologic gland with ultrasound guidance until the hypoechoic tissue becomes hyperechoic\textsuperscript{71}. Reports for the overall success rates for ethanol ablation have differed. The majority of PHPTH dogs had resolution of hypercalcemia with one treatment, however additional treatment may be needed\textsuperscript{66, 71}. Complications are related to extravasation of ethanol to affect the surrounding tissues and include coughing, dysphonia secondary to chemical injury to the recurrent laryngeal nerve, and continued hypercalcemia\textsuperscript{66, 70, 71}. 

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2.8.2 - Heat ablation

This technique utilizes radiofrequency to cause thermal necrosis. The abnormal parathyroid gland has to be larger than 3 mm to ensure adequate needle placement\textsuperscript{71}. Unlike ethanol ablation, extravasation does not occur with heat ablation and tends to spare the local blood supply\textsuperscript{116}. This procedure requires general anesthesia and a skilled ultrasonographer. In one study, 92\% of dogs had resolution of hypercalcemia with one treatment of percutaneous ultrasound-guided heat ablation\textsuperscript{71}. Another study reported a success rate of 73\%\textsuperscript{70}. Complications reported included cough, transient dysphonia, Horner’s syndrome and failure to resolve hypercalcemia\textsuperscript{71}.

2.8.3 - Parathyroidectomy

Surgical removal of the parathyroid glands has been the primary treatment for PHPTH in dogs for decades\textsuperscript{63}. Success rates, as defined by resolution of hypercalcemia, of 94-100\% have been reported\textsuperscript{63, 64, 67, 71}. Since adenomas do not tend to extend beyond the parathyroid capsule, the affected gland can be completely excised between the external parathyroid gland and the thyroid capsule. If the internal parathyroid gland cannot be isolated from the thyroid gland, a partial thyroidectomy with preservation of the ipsilateral external parathyroid gland can be elected. In situations where carcinoma is a more likely diagnosis, complete thyroparathyroidectomy is performed\textsuperscript{80}.

When comparing all three procedures, a statistical significance for success was noted comparing ethanol ablation to parathyroidectomy to treat PHPTH. Percutaneous ultrasound-guided ethanol ablation was the least successful of the treatment options and
also associated with the most complications. Compared to ethanol ablation, radiofrequency causes less damage to the surrounding tissues. Success rates of dogs treated with heat ablation had similar outcomes to dogs treated with parathyroidectomy. Time to hypercalcemia resolution after heat ablation treatment was significantly longer compared to ethanol treated dogs.

2.9 – Postoperative hypocalcemia

Postoperative hypocalcemia is the most common complication after parathyroidectomy. Between 33-40% of dogs became hypocalcemic following surgical parathyroidectomy and approximately 9.7-11% of the dogs developed clinical signs in two reports. Calcium concentrations start to decrease within 12 hours after surgery but can continue to decline over a period of 7 days. The decrease of ionized calcium occurs in a linear fashion in the first 24 hours after parathyroidectomy. There was no statistically significant predictive value of the slope of the decreased ionized calcium level during the first 24 hours after surgery and the development of hypocalcemia.

It is expected that hypersecreting parathyroid glands would cause negative feedback leading to inhibition and atrophy of the remaining non-pathologic parathyroid glands. Parathyroidectomy of the pathologic gland can lead to an acute and severe decrease in PTH levels resulting in hypocalcemia until the remaining parathyroid glands increase the synthesis and secretion of PTH. Although calcitriol is a hallmark treatment option of postoperative hypocalcemia, it causes direct inhibition to parathyroid cell
proliferation and suppression of PTH transcription and secretion. Traditional treatment for postoperative hypocalcemia is aimed at administering the end products of PTH action such as administering calcium salts or calcitriol.

Common clinical signs and symptoms of hypocalcemia include muscle twitching, muscle fasciculation, pawing or rubbing of the muzzle, weakness, lethargy, cardiac arrhythmias, tetany and death. Post-operative dogs should be kept under strict exercise restriction to limit the amount of calcium required for muscle metabolism and have serial ionized calcium measured at least every 12 hours for the first 2 to 4 days after surgery. In one study, 63% of dogs had the largest drop in calcium within the first 24 hours postoperatively and 30% of dogs had a decrease in calcium by 72 hours after surgery. Length of hospitalization is often clinician dependent, but recommendations for up to five days have been made to allow close monitoring to detect clinically important low concentrations of ionized calcium and immediate medical treatment if indicated.

Emergency management of hypocalcemia includes administering intravenous calcium gluconate 10% solution slowly at a dose of 0.5-1.5 ml/kg intravenously. If the clinical hypocalcemia is relatively mild (ie: pawing at face, tremors, muscle fasciculation), a constant rate infusion of calcium gluconate rather than a bolus can be utilized. It is imperative heart rate and rhythm is monitored with electrocardiogram, as bradycardia or disturbance to electrical conductance and death are possible with too rapid administration. Calcium gluconate should never be administered subcutaneously, even when diluted, as side effects include calcinosis cutis, severe pain, inflammation, abscess and necrosis. Once the dog is stable, oral supplementation of calcitriol and calcium
carbonate should be initiated. Calcitriol can be loaded at a dose of 10-15 ng/kg orally every 12 hours for 3-4 days, then decreased to 5 ng/kg orally every 12 hours can be administered to improve intestinal absorption of calcium and phosphorus. Oral supplementation of 25 mg/kg of elemental calcium every 12 hours can also be considered. Weekly evaluation of ionized calcium is necessary until steady state of serum calcium concentration has been reached at which point gradual tapering of calcium carbonate followed by tapering of calcitriol can be attempted. Target levels of ionized calcium that are desired should be high enough to ameliorate clinical signs but not high enough to blunt parathyroid gland activity which is just below the lower reference range or in the lower half of the reference range. The entire tapering process can take 3-6 months and some dogs may require lifelong supplementation. The administration of PTH (1-84) has recently been approved for the treatment of hypoparathyroidism in people. But this treatment option has not yet been reported for use in dogs.

Persistent and severe hypocalcemia after parathyroidectomy can be part of what has been termed “hungry bone syndrome” (HBS) and is seen with hypocalcemia, hypophosphatemia, hypomagnesemia and increased potassium levels. Occurrence of HBS is related to the increased skeletal usage of calcium, followed by removal of the hypersecreting parathyroid gland resulting in an immediate arrest of bone resorption in the face of continued bone formation. The duration of HBS is the time required for skeletal normalization and has been reported to last up to 9 months or longer in people. Pretreatment with bisphosphonates with or without calcitriol have shown promising
effects to either lessen the degree of HBS or completely attenuate postoperative hypocalcemia.  

2.10 - Study Rationale

Distinct pre-operative markers to predict postoperative hypocalcemia have not been identified in veterinary medicine. Post-operative hypocalcemia after parathyroidectomy is sometimes a life threatening condition depending on the nadir of circulating calcium that develops. Immediate intervention is necessary in some dogs to prevent death. Prolonged hospitalization is required for some dogs to allow time to gain sufficient control of circulating calcium at safe levels. Identifying predictive factors for the development of post-operative hypocalcemia would enhance monitoring and treatment recommendations in the post-operative period. Unlike previous PHPTH research in dogs, this study evaluates the relationship of PTH and vitamin D metabolites with the development of hypocalcemia after parathyroidectomy. To the authors’ knowledge, this is the first study to prospectively investigate the relationship of vitamin D metabolites in dogs with PHPTH at the time of initial diagnosis and at the time of calcium nadir.

The goal of this study was to identify preoperative parameters that may be utilized to predict development of postoperative hypocalcemia. We hypothesized that dogs with a >75% change between baseline and intraoperative PTH concentrations will be more likely to develop hypocalcemia in the immediate post-operative period. We also hypothesized that dogs with low preoperative serum 25-vitamin D or low preoperative
serum 1,25-vitamin D will be more likely to develop post-operative hypocalcemia in the immediate post-operative period.
Chapter 3: Materials and Methods

3.1 – Animals

The study protocol was approved by The Ohio State University-Institutional Animal Use Care and Committee. Dogs with naturally occurring PHPTH that were presented to The Ohio State University-Veterinary Medicine Center (OSU-VMC) were enrolled into the study with informed owner consent. Conformation of PHPTH was established following analysis of parathyroid hormone (PTH); parathyroid hormone related polypeptide (PTHrP) and ionized calcium (iCa) concentrations measured at the Michigan State University-Diagnostic Center for Population and Animal Health (DCPAH). The values from this panel were documented as the “Initial PTH value.”

In addition to a PTH/PTHrP/iCa panel, each enrolled dog had thoracic radiographs, serum biochemical profile, complete blood count and cervical ultrasound performed. Dogs that were presented with diagnostic tests (thoracic radiographs, DCPAH PTH results, complete blood count or biochemical profile) results from the referring veterinarian were accepted providing the diagnostic test was performed within 30 days to the time of surgery.

Overnight hospitalization prior to surgery with or without intravenous crystalloid fluid therapy was at the discretion of the surgeon responsible for the case. One dog was treated with two days of saline diuresis and initiation of calcitriol prior to undergoing parathyroidectomy based on the high preoperative ionized calcium level.
3.2 - Sampling protocol

Six ml’s of whole blood were obtained from each dog the day prior to parathyroidectomy. A portion of blood (0.7 ml) was used for ionized calcium and ionized magnesium analysis. Other analytes (Na, K, Cl, Mg, iCa, BUN, creatinine) were measured at the clinician’s discretion. The 0.7 ml of whole blood was transferred into a commercial pre-heparinized syringe and analyzed within 10 minutes of collection. The remaining volume was placed into a red top tube and allowed to clot. Centrifugation of the clotted sample was at 7000 rpm for 5 minutes. If at the end of the 5 minutes the sample necessitated more time in the centrifuge, an additional 5-minute spin at 7000 rpm was performed. The serum was transferred as 1 ml aliquot samples into Eppendorf tubes and stored in -80°C freezer until analysis.

3.2.1 - Sampling timeline

Each dog was sampled at the time of enrollment (Pre_O1) and the morning prior to parathyroidectomy (Pre_O2) from a peripheral vein. An intraoperative blood sample (IO1) was obtained through the central line placed into a jugular catheter 10 minutes after removal of the affected parathyroid. If more than one parathyroid gland was affected, an additional blood sample (IO2) was obtained 10 minutes after further parathyroidectomy. All intraoperative and post-operative blood samples were obtained through the central
venous catheter. The first post-operative blood sample (PO1) was obtained at 7pm the evening of surgery. Serial samples were obtained every 12 hours (ie: PO2 – 7am sample morning after surgery and PO3 – 7pm sample, etc) until the dog was discharged from the hospital. One dog was medically boarded after parathyroidectomy and sampling was discontinued at 14 days after parathyroidectomy.

3.2.2 – Electrolytes

All iCa and iMg samples were submitted to OSU-VMC clinical pathology laboratory for measurement. Heparinized blood samples were submitted immediately after collection and analyzed within 10 minutes of collection. Preoperative samples and postoperative samples were always submitted for a minimum of iCa and iMg panel. Other measurements that were performed include Na, K, Cl, BUN and creatinine on the NOVA machine.

3.2.3 – PTH

Batched frozen serum samples were submitted to Michigan State University-Diagnostic Center for Population and Animal Health (DCPAH) for radioimmunoassay PTH analysis. Frozen samples were shipped overnight in a Styrofoam shipping boxes with ice packs. Pre_O, IO, even day PO (ie: PO2, PO4) samples, 14 day postoperative samples and 90 days postoperative samples were submitted for analysis.
3.2.4 - Vitamin D Metabolites

Batched frozen serum samples were submitted to HeartLand Assays for 25-Vitamin D$_{3}$ and 1,25 Vitamin D$_{3}$ analysis. Frozen samples were shipped by overnight express on dry ice in a Styrofoam shipping box. Pre-O sample and the serum associated with ionized calcium nadir while hospitalized, 14 day postoperative sample and 90 days postoperative samples were submitted for analysis.

3.3 - General anesthesia and parathyroidectomy

Premedication was dosed at the discretion of the anesthesia service. A peripheral intravenous catheter was placed into the right or left cephalic vein. All dogs were placed under general anesthesia, which was maintained with isoflurane$^e$ or sevoflurane$^f$ and 100% oxygen. All patients received crystalloid fluid$^g$.

Dogs were positioned in dorsal recumbency and connected to a hemodynamic monitor$^h$ and standard surgical thermal support with a Bair Hugger$^i$. A standard ventral midline approach to the cervical region was performed on all dogs. Cervical exploration of both left and right thyroid and parathyroid glands were performed prior to parathyroidectomy. Based on surgeon preference, the affected parathyroid was removed with a combination of sharp and blunt dissection or LigaSure$^j$ device. Depending on surgeon preference, either a parathyroidectomy without peri-thyroid tissue was removed, or a thyroidectomy was performed with both the non-pathologic and non-pathologic parathyroid gland. One dog had a unilateral thyroidectomy and removed both the internal and external parathyroid gland on that side. One dog had three suspected parathyroid nodules removed by individual parathyroidectomies. Each parathyroid gland was
weighed (grams) and measured (mm). All tissues removed were submitted for histopathology.

A triple lumen central catheter was placed intraoperatively using the modified Seldinger technique. This catheter was placed immediately after parathyroidectomy and used for the intraoperative (IO) blood collection 10 minutes after parathyroidectomy. For the dog that had multiple (3) suspect parathyroid nodules removed, separate IO samples were collected for analysis 10 minutes after each parathyroidectomy. Routine closure was performed and each dog was admitted into the Intensive Care Unit after recovery from general anesthesia.

3.4 - Postoperative monitoring and hospitalization

All dogs recovered in the Intensive Care Unit after surgery for monitoring, nursing care, crystalloid fluid therapy and fentanyl continuous rate infusion or intermittent methadone bolus for pain control. Food and water were offered once the dog was sufficiently recovered from general anesthesia to allow swallowing.

Blood collection was through the central venous catheter based on the blood collection schedule. Standard operating procedure for blood collection from central venous catheter involved withdrawing 6 ml of blood into a heparinized syringe prior to collection of 6ml of blood for sample analysis. The 6ml of pre-sample blood was administered back to the dog through the central venous catheter followed by heparinized flush.
3.4.1 - Immediate perioperative period

Heparinized blood samples were obtained every 12 hours from the central venous catheter to monitor ionized calcium levels based on the previously described schedule and blood collection protocol. Additional iCa measurements were obtained for dogs that showed clinical signs of hypocalcemia between the planned sampling times.

3.4.2 - Two week and three month recheck

Dogs were returned 10-14 days after surgery for incision evaluation and suture removal. Heparinized blood was sampled at both the two week and three month recheck.

3.5 - Statistical analysis

Descriptive statistics were computed on SPSS (SPSS program) and reported as median (range). Non-parametric tests were used because of the small number of dogs in this study. Chi-squared analyses were used to evaluate relationships between binary variables such as hypomagnesemia (pre- and post-operative), hypovitaminosis D (pre- and post-operative) and the development of postoperative ionized hypocalcemia. Mann-Whitney U tests were used for analysis of continuous variables with binary variables such as percent change in PTH concentrations, length of hospitalization, pre-operative total and ionized calcium concentration, pre-operative vitamin D metabolite concentrations and the development of post-operative hypocalcemia. Spearman rho correlation analyses were used to evaluate length of hospitalization with percent PTH change.

All statistical analysis was performed with commercially available SPSS computer software. For all analysis, p<0.05 was considered significant.
3.6 - Footnotes

a pOHx STAT, Nova biomedical, Waltham, MA, USA

b Eppendorf tubes, Eppendorf North America, Hauppauge, NY, USA

c PTH radioimmunoassay , MSU-DCPAH, Lansing, MI, USA

d 25VitaminD and 1,25Vitamin D, HeartLand Assays, Ames, IA, USA

e Fluriso, MWI, Boise, ID, USA

f Sevoflurane, Baxter, Deerfield, IL, USA

g Lactate Plus, Nova Biomedical Corporation, Waltham, MA, USA

h Cardell MAX-12 DUO HD Multiparameter Monitor, Midmark Corp, Versailles, OH, USA

i Bair Hugger, 3M, St. Paul, MN, USA

j LigaSure Small Jaw Open Instrument, Medtronic, Minneapolis, MN, USA

k Triple lumen catheter, Arrow International Inc., Reading, PA, USA

l PlasmaLyte, Medline, Mundelein, IL, USA

m Fentanyl citrate injectable, Hospira Inj., Lake Forest, IL, USA

n Methadone, Xanodyne, Newport, KY

o SPSS, IBM SPSS Statistics software-version 21
Chapter 4: Results

Ten dogs with PHPTH were enrolled in the study. Median age of dogs with PHPTH was 10 years (range, 6-13 years). There were four mixed breed dogs and the remaining breeds consisted of Siberian husky, standard Schnauzer, Golden retriever, Redbone Coonhound, Border collie and Lhasa Apso. Seven dogs were spayed females and three were castrated male dogs. Median body weight was 28.6 kg (range, 4.7-46 kg).

4.1 - Clinical signs

Six dogs presented for polyuria and polydipsia. Of these six, two dogs also presented for weakness, lethargy and gastrointestinal signs such as anorexia and intermittent vomiting. One dog was presented for urethral obstruction associated with calcium oxalate urolithiasis. One dog had a history of weight loss, intermittent anorexia and tremors. Two dogs had no clinical signs and were diagnosed on routine blood work for annual examination.

4.2 - Parathyroid hormone

Five dogs had preoperative PTH concentrations above the reference range and five dogs had preoperative PTH concentrations within the reference range (reference range, 0.5-5.8 pmol/L). The median preoperative PTH concentration was 4.85 pmol/L (range, 1.3-24.6 pmol/L). Four of the five dogs that developed postoperative
hypocalcemia had elevated PTH concentrations at the time of diagnosis (p=0.016). (TABLE 1).

All PTH concentrations decreased into the normal ranges by 24 hours after parathyroidectomy and continued to remain normal during the perioperative hospitalization period (FIGURE 1). A statistical significance was found between the PTH level at the time of diagnosis and prediction of postoperative hypocalcemia (p=0.016; normocalcemic dogs: median, range 2.4 pmol/L, 1.3-5.9 pmol/L; hypocalcemic dogs: median, range 14 pmol/L, 3.8-24.6 pmol/L) (FIGURE 2). A statistical significance was found between the preoperative PTH concentration and prediction of postoperative hypocalcemia (p=0.032; normocalcemic dogs: median, range 2.8 pmol/L, 0.6-4.9 pmol/L; hypocalcemic dogs: median, range 21.1 pmol/L, 3.3-36.9 pmol/L) (TABLE 1) (FIGURE 3).

The percent change of circulating PTH concentration between the PTH level at diagnosis and intraoperative sample after parathyroidectomy was calculated. The median PTH percent change in all dogs’ was 77.6% (range, 53.8%-91.1%). Dogs that developed postoperative hypocalcemia had a significantly larger decrease in PTH concentration than dogs that did not develop hypocalcemia (p<0.008; normocalcemic dogs: median, range 70.8%, 53.8%-74.5%; hypocalcemic dogs: median, range 81.7%, 80.7%-91.1%) (TABLE 3) (FIGURE 4). A statistically significant difference was identified when evaluating the percent PTH change between preoperative and intraoperative samples and
the development of hypocalcemia. The median PTH percent change (preoperative to intraoperative) in all dogs was 81.2% (range, 16.7%-94.0%). Dogs that developed postoperative hypocalcemia had a significantly larger decrease in PTH concentration than dogs that did not develop hypocalcemia (p=0.032; normocalcemic dogs: median, range 75.0%, 16.7%-82.9%; hypocalcemic dogs: median, range 89.6%, 78.8%-94.0%) (TABLE 3) (FIGURE 5).

The percent change in PTH from the time of diagnosis to the intraoperative sample was significantly correlated to the post-operative ionized calcium nadir value (p=0.02, postoperative ionized calcium nadir median, range 4.97 mg/dl, 4.38-5.73 mg/dl). The percent change of PTH concentration was also significantly related to the need to prescribe post-operative 1,25-dihydroxyvitamin D and calcium supplementation (p<0.08). There was not a significant correlation between length of hospitalization after surgery and percent change in PTH concentration (p=0.10).

4.3 – Calcium

The median preoperative total calcium concentration was 14.3 mg/dl (range, 12.1-20.4 mg/dl). Female dogs (n=7) in this study had higher median preoperative serum total calcium of 15.5 mg/dl compared to male dogs (n=3) of 12.9 mg/dl. Median pre-operative total calcium in dogs that remained normocalcemic postoperatively was not significantly different than dogs that developed postoperative hypocalcemia (p=0.095; dogs that remained normocalcemic: median, range 13.3 mg/dl, 12.1-15.1 mg/dl; dogs that developed hypocalcemia: median, range: 15.6 mg/dl, 12.6-20.4 mg/dl) (TABLE 1).
Hypocalcemia was defined in this study as a concentration less than the reference range of ionized calcium.

The median preoperative ionized calcium concentration was 6.64 mg/dl (range, 5.96-9.22 mg/dl). The median pre-operative ionized calcium between dogs that developed and remained normocalcemic postoperatively and dogs that developed postoperative hypocalcemia approached significance (p=0.056; dogs that remained normocalcemic postoperative: median, range 6.25 mg/dl, 5.96-6.82 mg/dl; dogs that developed postoperative hypocalcemia: median, range: 8.02 mg/dl, 6.23-9.22 mg/dl). (TABLE 1).

All ionized calcium levels started to decrease starting the evening of surgery (FIGURE 6). Ionized calcium concentration returned to normal within 12 hours (two dogs), 24 hours (six dogs), and 36 hours (two dogs) after parathyroidectomy. Five dogs developed hypocalcemia during the immediate postoperative period; three of the five dogs developed subclinical hypocalcemia and two dogs developed clinical signs of hypocalcemia. The immediate postoperative period in this study is defined as the time the dog was hospitalized after parathyroidectomy. Calcium nadir values were reached within 48 hours postoperatively in four dogs, within 72 hours in three dogs, within 96 hours in two dogs and at 144 hours in one dog.

The percent change of ionized calcium from the preoperative value to the postoperative nadir was statistically significant (p = 0.008; dogs that remained normocalcemic dogs: median, range 15.9%, 9.28-18.3%; hypocalcemic dogs: median, range: 38.9%, 29.7-52.1%) (TABLE 3) (FIGURE 7).
4.4 – Phosphorus

Seven dogs had hypophosphatemia and three dogs had normal phosphorus values at the time of presentation. Normal phosphorus values were designated to be 3.2-8.1 mg/dl. The median preoperative phosphorus concentration was 2.6 mg/dl (range, 1.6-4.3 mg/dl). (TABLE 1).

4.5 – Magnesium

Two dogs had serum ionized magnesium levels below the reference range and eight dogs had serum magnesium concentrations within the reference range prior to surgery. Median preoperative serum ionized magnesium concentration was 1.18 mg/dl (range, 1.01-1.44 mg/dl). Median preoperative calcium:magnesium ratio for ten dogs was 6.04 (range 4.50-7.68). There was not a significant association between preoperative calcium:magnesium ratio and development of post-operative hypocalcemia (p=0.15). There was no association between calcium:magnesium ratio at nadir and the development of post-operative hypocalcemia (p=0.69). (TABLE 1).

4.6 - Alkaline phosphatase

Six dogs had elevated alkaline phosphatase (ALP) concentrations above the reference range, three dogs had normal pre-operative ALP and one dog had low ALP values. Normal ALP reference ranges 15-120 IU/ml. The median ALP concentration was
254 IU/ml (range, 40-2038 IU/ml). (TABLE 1). No dogs were treated with oral or injectable steroids at the time of surgery consultation or during the study period.

4.7 - Vitamin D

The reference range for 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D (1,25(OH)₂D) concentration in dogs was 54.9-97.9 ng/ml and 168.9-428 pg/ml respectively. The median preoperative 25(OH)D concentration was 33.3 ng/ml (range, 9.2-64.4 ng/ml) and the median 1,25(OH)₂D concentration was 242.85 pg/ml (range, 24.6-376.1 pg/ml). (TABLE 2). No significant difference was found between the preoperative 25(OH)D or preoperative 1,25(OH)₂D and the development of postoperative hypocalcemia (p=0.69 and p=0.42, respectively) (FIGURE 8 and FIGURE 9).

No statistical significance was found between preoperative 25(OH)D levels and postoperative calcium nadir (p=0.60; median, range: 29 ng/ml, range 10.3-67.9 ng/ml). No statistical significance was found between preoperative 1,25(OH)₂D levels and postoperative calcium nadir (p=0.803; median, range: 128.5 pmol/ml, 65.9-271.2 pmol/ml) (TABLE 3). No statistical significance was found between preoperative vitamin D levels and postoperative 25(OH)D nadir (p=0.42) and 1,25(OH)₂D nadir (p=0.84).
4.8 - Cervical ultrasonography

All dogs had cervical ultrasounds performed. Fifteen presumed enlarged parathyroid glands were detected preoperatively in ten dogs. Seven dogs had uniglandular disease with three localized to the left parathyroid glands and four localized to the right side. One dog had multiglandular enlargement of all parathyroid glands, one dog had bilaterally enlarged hypoechoic parathyroid nodules with a larger right hypoechoic nodule within the parenchyma of the right thyroid gland. Another dog had two hypoechoic nodules within the parenchyma of the right thyroid gland.

4.9 - Surgical findings

Seven dogs had a parathyroidectomy of the enlarged parathyroid gland and three dogs had the enlarged parathyroid gland removed by unilateral thyroidectomy. Not including the normal sized parathyroid gland in the dogs that had a thyroidectomy, a total of 12 enlarged suspect parathyroid glands were removed in 10 dogs. Four of 12 were left parathyroid glands and 8 were from the right side. One dog had three abnormal hypoechoic nodules identified on ultrasound and all three were removed during surgery.

4.10 – Histopathology

Ten of the 12 presumed parathyroid glands were confirmed to be parathyroid glands with the remaining two being consistent with nodular thyroid hyperplasia. Two of the 10 parathyroid glands were consistent with hyperplasia (20%), six were parathyroid adenomas (60%) and the remaining two glands were carcinoma (20%). In one dog with
three hypoechoic nodules identified on ultrasound, one was parathyroid carcinoma, one was thyroid carcinoma and the last gland was thyroid nodular C-cell hyperplasia.

4.11 – Postoperative hypocalcemia treatment

All dogs that developed clinical hypocalcemia (n=5) were immediately treated with a bolus of intravenous calcium gluconate solution and continued on 1,25-dihydroxyvitamin D and oral calcium carbonate. One dog that developed clinical ionized hypocalcemia initially presented with an ionized calcium concentration of 8.17 mg/dl, total calcium 20.4 mg/dl, and was treated for two days prior to parathyroidectomy with intravenous saline diuresis and oral 1,25-dihydroxyvitamin D twice a day. This dog developed neurologic signs that lead to seizure activity when the ionized calcium had decreased post operatively but was still above the normal reference range. The other dog that developed clinical hypocalcemia had a preoperative ionized hypercalcemia of 9.22 mg/dl, total calcium of 16 mg/dl, and was seen to paw the face when the ionized calcium was 4.42 mg/dl. All dogs that developed postoperative hypocalcemia regardless of lack of clinical signs received oral supplementation of calcitriol or calcium carbonate. The exact decision as whether one or both oral supplementation was used was based on the primary surgeons discretion.

4.12 - Length of hospitalization

The median length of hospitalization after surgery was 8 days (range, 1.5-9.5 days). One dog was discharged two days postoperatively, three days after surgery (four
dogs), four days after surgery (three dogs), nine days after surgery (one dog) and one dog was discharged 10 days after surgery. The two dogs that were hospitalized for 9 and 10 days after parathyroidectomy both developed clinical hypocalcemia.
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<th>Preop tCa (mg/dl)</th>
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Table 2 - Preoperative and postoperative data (1,25(OH)D, 25(OH)D and PTH) values for dogs that remained normocalcemia and developed hypocalcemia after parathyroidectomy.

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*1,25VD values from 12 hours prior to calcium nadir

**25VD values from 12 hours prior to calcium nadir

^PTH was not submitted at that time point. The recorded value is an average of the PTH concentration from 12 hours prior and 12 hours after.
**Figure 1**: Trend of PTH (pmol/L) concentration per dog while hospitalized and follow-up at 10-14 days (Day 14) and 90 days (Day 90) after parathyroidectomy. Day 0 = Morning of surgery prior to parathyroidectomy.
Figure 2: PTH (pmol/L) level at the time of diagnosis and postoperative hypocalcemia.

Normocalcemic dogs: median, range 2.4 pmol/L, 1.3-5.9 pmol/L; hypocalcemic dogs: median, range 14 pmol/L, 3.8-24.6 pmol/L. (p = 0.016).
Figure 3: Preoperative PTH (pmol/L) level and postoperative hypocalcemia.

Normocalcemic dogs: median, range 2.8 pmol/L, 0.6-4.9 pmol/L; hypocalcemic dogs: median, range 21.1 pmol/L, 3.3-36.9 pmol/L. (p = 0.032).
Table 3 – Percent PTH change, percent calcium change and vitamin D data values

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<th>Normocalcemia (Range)</th>
<th>Hypocalcemia (Range)</th>
<th>p value</th>
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<td>%PTH change (initial to intraoperative)</td>
<td>77.6% (53.9-91.1%)</td>
<td>70.8% (53.8-74.5%)</td>
<td>81.7% (80.7-91.1%)</td>
<td>&lt;0.008</td>
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<tr>
<td>%PTH change (preoperative to intraoperative)</td>
<td>81.2% (6.7-94.0%)</td>
<td>75.0% (16.7-82.9%)</td>
<td>89.6% (78.8-94.0%)</td>
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<td>%iCa-nadir</td>
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<td>15.9% (9.28-18.3%)</td>
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Figure 4: Percent PTH change (initial PTH at diagnosis and intraoperative PTH) and the development of hypocalcemia. The median PTH percent change was 77.6% (range, 53.8%-91.1%). Normocalcemic dogs: median, range 70.8%, 53.8%-74.5%; hypocalcemic dogs: median, range 81.7%, 80.7%-91.1%. (p < 0.008).
Figure 5: Percent PTH change (preoperative PTH and intraoperative PTH) and the development of hypocalcemia. The median PTH percent change was 81.2% (range, 16.7%-94.0%). Normocalcemic dogs: median, range 75.0%, 16.7%-82.9%; hypocalcemic dogs: median, range 89.6%, 78.8%-94.0%. (p = 0.032).
Figure 6: Trend of ionized calcium during the immediate postoperative period. Day 0 = Morning of surgery prior to parathyroidectomy. Ionized calcium concentration returned to normal ranges within 12 hours (n=2), 24 hours (n=6), and 36 hours (n=2) after parathyroidectomy.
Figure 7: Percent change in ionized calcium (initial iCa to nadir iCa) and the development of postoperative hypocalcemia. *Normocalcemic dogs*: median, range 15.9%, 9.28-18.3%; *hypocalcemic dogs*: median, range: 38.9%, 29.7-52.1%. (p = 0.008).
**Figure 8**: Preoperative 25(OH)D and development of postoperative hypocalcemia.

*Normocalcemic dogs*: median, range 44.7 ng/ml, 21.6-64.4 ng/ml; *hypocalcemic dogs*: median, range 31 ng/ml, 9.2-45.4 ng/ml. (p = 0.69).
**Figure 9:** Preoperative $1,25(\text{OH})_2\text{D}$ and development of postoperative hypocalcemia.

*Normocalcemic dogs:* median, range 229.4 pg/ml, 187.4-304.2 pg/ml; *hypocalcemic dogs:* median, range 265.9 pg/ml, 24.6-376.1 pg/ml. ($p = 0.42$).
Chapter 5: Discussion

Primary hyperparathyroidism in dogs can be associated with both subclinical and life threatening hypocalcemia following surgical excision. The current literatures on postoperative hypocalcemia are retrospective in nature with various conclusions. This is the first prospective study accruing data in the perioperative and postoperative time setting, including vitamin D metabolite levels with conclusions that are applicable in a clinical setting.

The age and weight distribution of the dogs in this study were consistent with previous reports indicating PHPTH tends to be a disease of older, medium-large sized dogs. An autosomal dominant inheritance pattern has been identified in the Keeshonden dog breed with an odds ratio of developing PHPTH of 50.7. Results of other studies have supported the genetic link that Keeshonden dogs are predisposed, however, there were no Keeshonden dogs enrolled in this study which may be reflective of a lack of breed popularity within the general public, small sample size or regional variation in populations of dogs.

PHPTH in people has a higher sex predisposition for women with a ratio of approximately 2.8:1 to 3.3:1, occurring in post-menopausal women. Estrogen has a known protective effect against PTH on bone resorption.
which may be why men were more commonly asymptomatic or symptomatic for kidney stones whereas women more commonly had bone related diseases such as osteoporosis.\textsuperscript{119} Men were also noted to have significantly elevated preoperative serum total calcium and parathyroid hormone concentrations.\textsuperscript{119} No sex predisposition has been reported in dogs.\textsuperscript{62, 64, 67, 69} A greater number of female dogs than male dogs were reported in this study with a higher median preoperative serum total calcium 15.5 mg/dl and 12.9 mg/dl, respectively, and the six highest PTH levels at diagnosis. The significance of this is unknown and should be evaluated further in future studies with higher case numbers.

The clinical signs reported in this study were consistent with previous veterinary PHPTH publications where the most common presenting sign was polyuria and polydipsia.\textsuperscript{62, 65} The percentage of dogs diagnosed with concurrent cystic calculi (20\%) in this study was similar to other publications that reported 24\% of dogs to have cystic calculi at the time of referral\textsuperscript{62}, with one dog being presented on emergency for urethral obstruction. The cystolith composition was calcium oxalate in both cases. The other clinical signs that were seen in this study are categorized into gastrointestinal or neurologic signs and tend to be less commonly reported by owners than polyuria and polydipsia.\textsuperscript{62, 64, 65} Twenty percent of dogs in this study were asymptomatic for PHPTH, which is less compared to other publications.\textsuperscript{62, 64, 65}

Dogs with PHPTH tend to have decreased serum phosphorus that is attributable to PTH-induced inhibition of renal tubular phosphorus resorption.\textsuperscript{62} Seven (70\%) dogs had hypophosphatemia and three (30\%) dogs had normal phosphorus levels in this study.
This was a similar proportion to the finding of a larger retrospective study but not consistent with the findings of another study. Interestingly, the dog with the highest (4.3 mg/dl) preoperative phosphorus value was mildly azotemic and developed severe azotemia at the three-month postoperative recheck. This was similar to three of the PHPTH dogs reported in a previous study that had higher phosphorus levels and renal insufficiency. A correlation with preoperative phosphorus level and development of postoperative hypocalcemia has not been documented.

Alkaline phosphatase may be elevated in dogs with PHPTH due to increased activity of the bone isoenzyme relating to bone turnover. PTH has effects on osteoclasts to resorb bone in order to mobilize and increase circulating calcium. Previously, an elevated alkaline phosphatase had been documented in some dogs with PHPTH. Seven (70%) dogs had elevated alkaline phosphatase and three (30%) dogs had alkaline phosphatase within normal ranges. None of the dogs were treated with steroids at the time of surgery consultation or during the study period. PHPTH is a disease of older dogs, however, and it is possible that concurrent hepatic, endocrinopathies or administration of medications have contributed to elevation of alkaline phosphatase. It has been reported in people that excess PTH causes increased alkaline phosphatase and is also associated with radiographic evidence of bone resorption. A duel-energy X-ray absorptiometry (DEXA) scan would be necessary to evaluate for bone mineral density. In addition, there were no correlations between preoperative alkaline phosphatase and PTH concentrations in the current study.
Postoperative hypocalcemia is the most common complication after parathyroidectomy. In previous studies, 33-40% of dogs became hypocalcemic following surgical parathyroidectomy and approximately 9.7-11% of the dogs developed clinical signs\textsuperscript{69, 71}. The percentage of dogs that developed hypocalcemia in this study was higher than previously reported. The mechanism for postoperative hypocalcemia after parathyroidectomy is multifactorial and not fully understood. It likely involves suppression of the normal parathyroid glands, sudden cessation of PTH mediated bone resorption and increased uptake of calcium into remineralizing bone, decreased intestinal absorption of calcium through decreased conversion of 25(OH)D to 1,25(OH)\textsubscript{2}D. Other causes include surgical hypoparathyroidism, which is typically transient but may be related to disruption of the blood supply to the remaining parathyroid glands.

Prolonged exposure of circulating PTH may alter the set-point mechanism resulting in tissue resistance to PTH. This has been reported in people with chronic kidney disease and primary hyperparathyroidism\textsuperscript{121, 122}. People with chronically elevated PTH concentration may have decreased sensitivity to 1,25(OH)\textsubscript{2}D as evident by decreased density of the vitamin D receptor\textsuperscript{123, 124}. In the postoperative period, this alteration of PTH-calcium set-point may necessitate additional PTH to generate a response from the target tissues and may lead to postoperative secondary hyperparathyroidism\textsuperscript{7, 125}.

Preoperative factors previously reported in association with postoperative calcium nadir include a history of weakness, PTH concentration higher than reference range and high serum BUN concentration\textsuperscript{69}. That study also found a significant negative correlation
between age and post-operative calcium nadir, old dogs had lower serum total calcium nadir concentrations when compared to young dogs\textsuperscript{69}. The negative feedback caused by chronic hypercalcemia the result of hypersecreting parathyroid glands, leads to atrophy of the non-pathologic parathyroid glands. In this situation, following parathyroidectomy of the pathologic gland, a transient hypoparathyroid state would occur, leading to continued decrease of calcium levels, but studies have not routinely reported PTH concentrations following parathyroidectomy.

Feldman et al\textsuperscript{73} suggested that PHPTH dogs with a higher preoperative serum total calcium level (>14 mg/dl) are at a greater risk of developing postoperative hypocalcemia. The results of our study did not show a statistical difference between preoperative total calcium concentration and developing postoperative hypocalcemia. The total calcium value is influenced by circulating protein concentration and albumin concentration that were not assessed in this study. It is also known that total calcium is a less sensitive parameter than ionized calcium as an assessment of circulating calcium levels\textsuperscript{1}. We did not find a significant correlation between preoperative ionized calcium and preoperative PTH level in this study as expected in animals with parathyroid tumors in which down regulation by calcium is lacking or absent. Preoperative ionized calcium was not significantly correlated with developing postoperative hypocalcemia in a retrospective study\textsuperscript{65}. Our data showed a trend (p=0.056) towards higher pre-operative ionized calcium in dogs that developed hypocalcemia post-operatively compared to dogs that did not. The two dogs that developed clinical hypocalcemia had the highest pre-operative total and ionized calcium levels. This may reflect prolonged atrophy of the
remaining parathyroid glands, recruitment of calcium for bone remineralization, or continued polyuria with renal calcium excretion. When evaluating the percent change of calcium between preoperative ionized calcium and the ionized calcium nadir after surgery, a significant association (p=0.008) was found in dogs that became hypocalcemic. Ionized calcium concentrations started to decrease in all dogs on the evening post surgery. In eight of 10 dogs ionized calcium normalized within 24 hours after surgery and in two dogs ionized calcium normalized by 36 hours after surgery. The decrease of ionized calcium occurred in a linear fashion in the first 24 hours after parathyroidectomy in our study as was reported in another study. The rate of decline in the first 24 hours did not predict the development of hypocalcemia in the same study. The time to ionized calcium nadir was variable in our study. Four dogs reached nadir 48 hours after surgery, three dogs within 72 hours after surgery, two in 96 hours after surgery and the longest time until nadir was 144 hours. Dogs in our study were discharged at varying days after surgery based on the primary clinician discretion. It is possible that dogs that were discharged relatively soon after surgery had reached a new nadir at home.

All elevated PTH concentrations decreased into the normal by 24 hours after parathyroidectomy and all PTH concentrations continued to remain in the normal range during the time of hospitalization after surgery. Other studies have also demonstrated that PTH concentrations decrease within the first 24 hours after surgery and reach a plateau at varying times afterwards. Unlike other reports that did not find a correlation between preoperative PTH concentration and prediction of postoperative hypocalcemia, dogs that developed postoperative hypocalcemia had higher PTH concentrations at
diagnosis compared to dogs that did not develop hypocalcemia postoperatively. One study found similar results with higher than reference range PTH to be associated with low postoperative total calcium\textsuperscript{69}.

The introduction of intraoperative parathyroid hormone assay has altered surgical options and improved the success of parathyroidectomy in people with PHPTH. The assay can measure PTH level in plasma and since the hormone has a short-half life, it decreases rapidly when all hypersecreting tissue is excised\textsuperscript{6}. A decrease in PTH concentration of >50%, from the pre-surgical sample ten minutes after parathyroidectomy is indicative of successful removal of all autonomously functioning parathyroid tissue in people\textsuperscript{94, 126}. Focused cervical surgery with minimally invasive approaches has improved postoperative cosmesis in people requiring cervical interventions by allowing discrete incisions\textsuperscript{126}. Although cosmesis is not usually a significant concern in dogs, intraoperative PTH assay has been used to confirm successful parathyroidectomy in dogs\textsuperscript{67, 68}.

In our study, the percent change of PTH between the sample at the time of diagnosis and the intraoperative sample was correlated to development of hypocalcemia after parathyroidectomy. Dogs with a percent change of PTH greater than 75% were significantly more likely to develop postoperative hypocalcemia compared to dogs that did not. The degree of normal parathyroid gland atrophy may be related to duration of PHPTH or perhaps related to the severity of disease. The only stimulus of PTH transcription and secretion is hypocalcemia. This suggests that individuals with parathyroid gland atrophy would require a period of hypocalcemia in order to return a
non-functional parathyroid gland to secreting PTH. Unfortunately, significant hypocalcemia is detrimental and can be associated with fatality. Interestingly, 1,25(OH)₂D, which is the most active natural metabolite of vitamin D and a commonly used medication in postoperative PHPTH individuals, inhibits parathyroid cell proliferation and thus inhibits PTH secretion⁴³. A correlation between parathyroid gland size and amount of PTH secretion has been established in people¹²⁷,¹²⁸. Literature about the relationship between parathyroid gland size and amount of PTH produced has not been reported in the veterinary medicine. Further evaluation of parathyroid gland mass, PTH concentration, and severity of disease should be evaluated in future studies.

Cervical ultrasonography is an efficient and non-invasive method to examine parathyroid gland size and determination of the number of glands affected. Previous reports have identified ultrasonography as having good correlation with surgical findings in dogs⁶⁴,⁶⁵,¹⁰⁸. All dogs of this study received cervical ultrasonography evaluations by board certified radiologists, or under the direct supervision of a board certified radiologist, between two specialty practices. In terms of laterality, nine of the 10 dogs had ultrasound reports that identified the correct location of the largest hypoechoic. Previous reports identified an agreement of 75.9% between ultrasonographic results regarding laterality of the abnormal parathyroid gland and surgical findings⁶⁹. This further supports the notion that success of ultrasonography is subjective and operator dependent.

Clinical information about vitamin D metabolites in veterinary medicine has largely focused on dogs and cats with chronic kidney disease¹²⁹,¹³⁰,¹³¹ and in dogs with
intestinal disease and hypocalcemia associated with hypovitaminosis D. Neither 25(OH)D or 1,25(OH)₂D concentrations prior to surgery predicted the development of post operative hypocalcemia. Also, the concentrations of 25(OH)D and 1,25(OH)₂D at the time of calcium nadir failed to predict those that developed hypocalcemia from those that did not. The concentrations of 25(OH)D and 1,25(OH)₂D did not differ between preoperative measurement and measurements at the time of calcium nadir. It is interesting to note that 25(OH)D concentrations were lower than the reference range at the time of diagnosis even though it did not predict the development of hypocalcemia.

Regulation of calcium homeostasis is one of the major functions of 1,25(OH)₂D, which targets the intestines, kidneys, bone and the parathyroid glands. It is well known that people with PHPTH and concurrent 25(OH)D deficiencies are associated with more severe PHPTH disease as seen with higher calcium, higher alkaline phosphatase, higher baseline PTH and larger parathyroid glands⁵⁷,⁸⁴. In this study, a significant correlation was not found comparing preoperative 25(OH)D or 1,25(OH)₂D levels and developing postoperative hypocalcemia. However, all 10 dogs in the study had low preoperative 25(OH)D levels and nine dogs had normal preoperative 1,25(OH)₂D and one dog had low preoperative 1,25(OH)₂D levels. Possible reasons for low 25(OH)D in PHPTH can be related to increased conversion of 25(OH)D to 1,25(OH)₂D, decreased vitamin D absorption, decreased conversion to 25(OH)D in the liver, increased 25(OH)D catabolism or increased storage in adipose tissue⁴⁶,⁵⁷.

Alternatively, chronic hypovitaminosis D may contribute to development of PHPTH by inducing stimulation of PTH secretion to increase circulating 1,25(OH)₂D. It
has also been suggested that chronic vitamin D deficiency may accelerate parathyroid gland growth. It is known that dogs with vitamin D deficiency have difficulties increasing the amount of circulating calcium even in the presence of elevated PTH production. A type II statistical error cannot be ruled out and additional studies with larger cohorts of dogs should be performed. The reference ranges established for vitamin D metabolites in dogs is variable. The reference range selected for use in this study was based on that for 10 healthy companion dogs measured at the same lab and recently reported in the veterinary literature. All dogs in our study were fed a commercial dog food that was not detailed as to nutritional D (cholecalciferol) intake. A difference in vitamin D absorption may occur based on sex and breed of dog resulting in varying concentrations of 25(OH)D following absorption. Additional research to better understand vitamin D metabolism is warranted for dogs with PHPTH. A specific goal should be to determine if low 25(OH)D concentrations as noted in our pre-operative dogs are associated with the development of PHPTH and its severity.

This is the first prospective study analyzing PTH, 25(OH)D and 1,25(OH)₂D concentrations dogs with PHPTH undergoing surgical treatment. The pitfalls of using different analyzers and non-standardized sample handling protocols used in previous studies were avoided in the present study. All ionized calcium samples in this study were handled based on a strict protocol and all measurements were performed on the same machine. PTH as well as 25(OH)D and 1,25(OH)₂D were measured by one laboratory specializing in these measurements. This study provided time matched plasma ionized
calcium and serum PTH samples and time matched vitamin D metabolites which many retrospective studies.
Chapter 6: Conclusion

Post-operative hypocalcemia after parathyroidectomy can be life threatening with significant financial responsibilities. Identifying predictive factors for postoperative hypocalcemia would improve patient care. In this study, hypocalcemia developed in five of 10 dogs in our study, but only two of those five dogs developed recognizable clinical signs. Based on the results of this study, dogs with a 75% or greater decrease of PTH between the diagnostic sample or greater than 80% change in PTH from the pre-operative to the intra-operative PTH sample had a significantly higher risk of developing post-operative hypocalcemia. The magnitude of the hypercalcemia based on total calcium before surgery did not predict which dogs would develop hypocalcemia or clinical signs of hypocalcemia. The magnitude of ionized hypercalcemia approached but did not achieve statistical significance for the development of postoperative hypocalcemia.

Neither 25(OH)D or 1,25(OH)₂D concentrations predicted which dogs developed post-operative hypocalcemia. Reasons for the low preoperative 25D concentrations are not apparent.

Continued research is necessary to better understand the disease of PHPTH and causes of prolonged postoperative hypocalcemia. In the clinical patient, the gold standard of PHPTH monitoring should involve measuring intraoperative PTH to confirm excision
of hyperfunctioning parathyroid glands and predict the likelihood of postoperative hypocalcemia. Vitamin D panels should also be evaluated prior to parathyroidectomy as dogs with PHTPH may have low calcidiol values that may benefit from pretreatment prior to parathyroidectomy.
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