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METABOLISMO MINERAL EN GATOS
MINERAL METABOLISM IN CATS

CARMEN MARÍA PINEDA MARTOS
CÓRDOBA, 2014

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METABOLISMO MINERAL EN GATOS

MINERAL METABOLISM IN CATS

MEMORIA DE TESIS DOCTORAL presentada por **CARMEN MARÍA PINEDA MARTOS,** Licenciada en Veterinaria**, para optar al grado de DOCTORA**

DOCTORAL THESIS presented by **CARMEN MARIA PINEDA MARTOS,** Licenciada en Veterinaria**, for the degree of DOCTOR**

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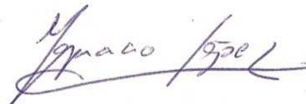
INFORMA:

Que doña CARMEN MARÍA PINEDA MARTOS, Licenciada en Veterinaria, ha realizado bajo mi dirección en el Departamento de Medicina y Cirugía Animal de la Universidad de Córdoba, el trabajo titulado: *"Mineral Metabolism in Cats (Metabolismo Mineral en Gatos)"*, y que a mi criterio dicho trabajo reúne los méritos suficientes para optar al Grado de Doctora en Veterinaria.

Y para que así conste y surta los efectos oportunos, firmo el presente informe en Córdoba, a doce de Mayo de dos mil catorce.



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TÍTULO DE LA TESIS: MINERAL METABOLISM IN CATS (Metabolismo Mineral en Gatos)

DOCTORANDO/A: CARMEN MARÍA PINEDA MARTOS

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La doctoranda Carmen María Pineda Martos está colaborando activamente con nuestro grupo de investigación desde el año 2009. Dicha colaboración se inició con su incorporación al Máster en Medicina, Sanidad y Mejora Animal de la Universidad de Córdoba durante el curso 2008-2009. Durante este periodo inició una labor investigadora que junto con la Licenciatura en Veterinaria encontró su continuación académica en los estudios de Doctorado. Su proyecto de Tesis Doctoral está encuadrado en el Programa de Doctorado de la Universidad de Córdoba correspondiente al área de Biociencias y Ciencias Agroalimentarias (P.D. con mención de calidad), estando a su vez adscrito a la línea de investigación Fisiopatología del Metabolismo Mineral.

La presente Tesis Doctoral, llevada a cabo bajo nuestra supervisión y dirección, se ha desarrollado durante el periodo comprendido entre 2010 y 2014. Durante dicho periodo la doctoranda ha mostrado una gran dedicación e interés en las tareas de investigación asignadas. De igual modo, ha sido capaz de realizar una labor de investigación con gran validez científica, fruto de la cual dicha Tesis Doctoral ha originado varias publicaciones en revistas de alto interés científico dentro del ámbito de la veterinaria.

Además con objeto de completar su formación y profundizar en el estudio del metabolismo mineral felino, la doctoranda realizó una estancia de tres meses en la Universidad de Bristol (Reino Unido).

El trabajo realizado en esta Tesis Doctoral ha generado la siguiente producción científica:

a) Publicaciones en revistas científicas indexadas:

Pineda C, Aguilera-Tejero E, Raya AI, Diez E, Rodriguez M, Lopez I. Feline parathyroid hormone: validation of hormonal assays and dynamics of secretion. *Domest Anim Endocrinol* 2012; 42: 256-264.

Pineda C, Aguilera-Tejero E, Guerrero F, Raya AI, Rodriguez M, Lopez I. Mineral metabolism in growing cats: changes in the values of blood parameters with age. *J Feline Med Surg* 2013; 15: 866-871.

Pineda C, Aguilera-Tejero E, Raya AI, Guerrero F, Rodriguez M, Lopez I. Assessment of calcitonin response to experimentally induced hypercalcemia in cats. *Am J Vet Res* 2013; 74: 1514-1521.

Pineda C, Aguilera-Tejero E, Raya AI, Montes de Oca A, Rodriguez M, Lopez I. Effect of calculolytic diets on feline mineral metabolism. *In Press*.

b) Comunicaciones científicas en Congresos Internacionales:

Pineda C, Navarro B, Viejo EL, Perales A, Raya A, Diez E, Aguilera-Tejero E, Lopez I. Blood parameters related to mineral metabolism along the first year of life in european shorthair cats. 21st EVCIM-CA Congress (European College of Veterinary Internal Medicine-Companion Animals). 08-10 Septiembre de 2011. Sevilla, España.

Pineda C, Aguilera-Tejero E, Raya AI, Diez E, Lopez I. Dynamics of PTH secretion in response to hypo- and hypercalcemia in healthy cats. 45th European Veterinary Conference-Voorjaarsdagen (Netherlands Association for Companion Animal Medicine). 05-07 Abril de 2012. Ámsterdam, Países Bajos.

c) Comunicaciones científicas en Congresos Nacionales:

Pineda C, Aguilera-Tejero E, Raya AI, Guerrero F, Diez E, Montes de Oca A, Lopez I. Secreción de calcitonina en gatos: respuesta a hipercalcemia. VII Congreso Andaluz de Veterinarios especialistas en animales de compañía (Consejo Andaluz de Colegios Oficiales de Veterinarios). 04-05 Noviembre de 2011. Almería, España.

Pineda C. Secreción de calcitonina en gatos: respuesta a hipercalcemia. I Congreso Científico de Investigadores en Formación en Agroalimentación de la eidA3 y II Congreso Científico de Investigadores en Formación de la Universidad de Córdoba (Postgrado y Formación Continua-Universidad de Córdoba). 08-09 Mayo de 2012. Córdoba, España.

d) Comunicaciones científicas en Cursos de Formación Permanente Universitaria:

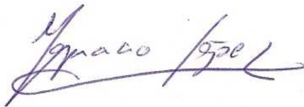
Pineda C, Navarro B, Viejo EL, Perales A, Raya AI, Guerrero F, Diez E, Aguilera-Tejero E, Lopez I. Evolución de los principales parámetros del metabolismo mineral en gato común europeo durante el primer año de vida. Curso de Formación Permamente: "Investigación en Veterinaria y Ciencia y Tecnología de los Alimentos" (Decanato de la Facultad de Veterinaria-Universidad de Córdoba). 07-11 Noviembre de 2011. Córdoba, España.

Además de trabajar en el desarrollo y progreso de su trabajo de Tesis Doctoral, la doctoranda ha participado activamente en los diversos proyectos de investigación que llevamos a cabo en nuestra unidad, figurando entre los autores de numerosas comunicaciones y artículos científicos.

Por todo lo anteriormente expuesto, se autoriza la presentación de la Tesis Doctoral.

Córdoba, 12 de MAYO de 2014

Firma del/de los director/es



Fdo.: Dr. IGNACIO LÓPEZ VILLALBA   Fdo.: Dr. ESCOLÁSTICO AGUILERA TEJERO

A mis padres
A mi hermana

A lo largo del proceso de elaboración de la presente Tesis Doctoral ha habido muchas personas que de un modo u otro han colaborado para que finalmente un proyecto incipiente y cauteloso haya conseguido consolidarse y pisar tierra firme. Tras varios años de esfuerzo y trabajo es realmente grato conseguir que todo aquello que has estado sembrando finalmente consiga dar fruto. A través de estas líneas quiero expresar mi agradecimiento y gratitud a todos aquellos que han formado parte de este trabajo y han contribuido para que finalmente se consiga el propósito establecido.

Al Dr. Ignacio López Villalba, por su magnífica labor como director de esta Tesis Doctoral, por su apoyo, confianza y ayuda inestimable siempre que la he necesitado. Por su ambición contagiosa de esfuerzo, aprendizaje y superación como profesional día a día. Agradecerle el haberme brindado la oportunidad de trabajar a su lado todo este tiempo. Por los buenos y malos momentos y porque siempre he podido contar con él, más que como director como un amigo.

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A mis compañeros del Departamento de Medicina y Cirugía Animal y del Hospital Clínico Veterinario de la Universidad de Córdoba.

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Al Servicio de Animales de Experimentación – Animalario de la Universidad de Córdoba.

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A los animales que han formado parte de este estudio, mis gatos de tesis.

A mis amigos, por estar siempre presentes, por comprender mi trabajo y apoyarme en todo momento.

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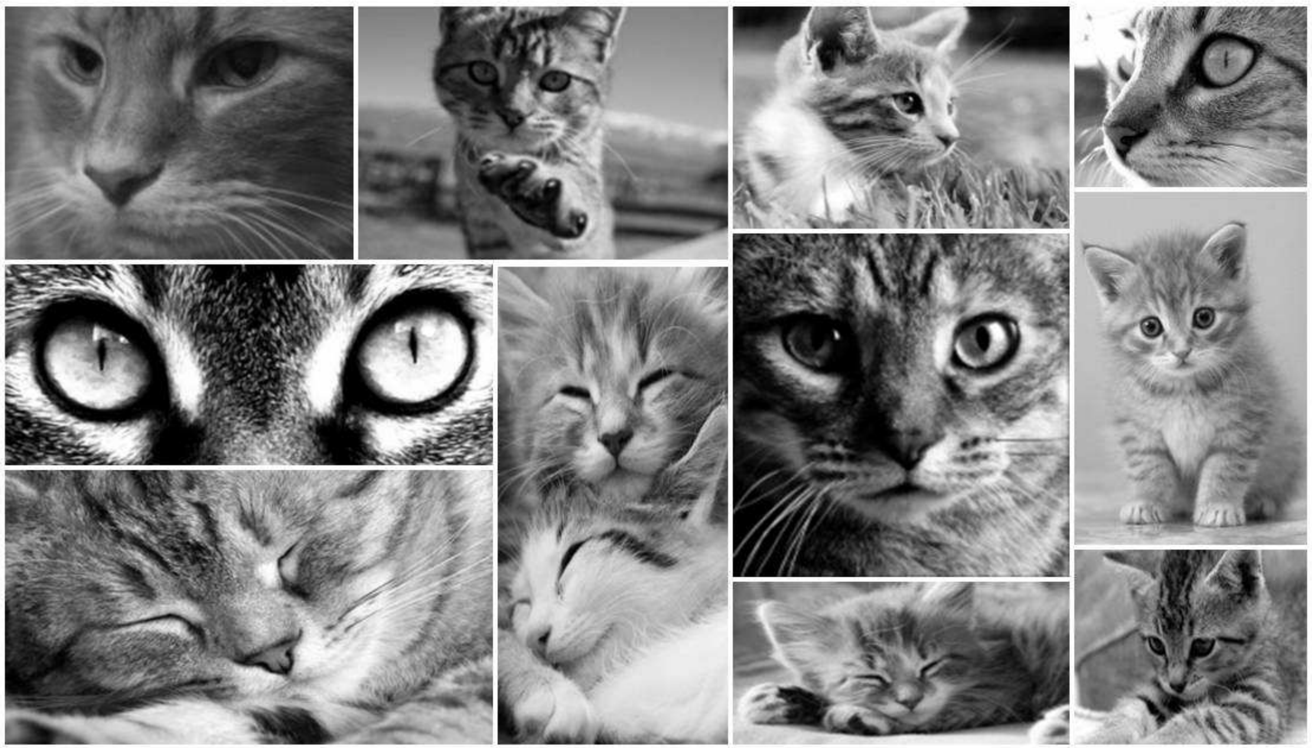
A todos y cada uno de vosotros, muchísimas gracias.



Mineral Metabolism in Cats

Doctoral Thesis
BY COMPENDIUM OF PUBLICATIONS
AND WITH INTERNATIONAL MENTION

CARMEN MARIA PINEDA MARTOS
CORDOBA, 2014

LIST OF ABBREVIATIONS

Below, a list of abbreviations used throughout the text is presented:

1,25-dihydroxyvitamin D_3	calcitriol
25-hydroxyvitamin D_3	calcidiol
cAMP	3',5'-cyclic monophosphate
CKD	chronic kidney disease
C-PTH	carboxyl-terminal parathyroid hormone
CT	calcitonin
CT-Ca curve	calcitonin-calcium curve
FE	fractional excretion
FGF	fibroblast growth factor
FLUTD	feline lower urinary tract disease
iCa	ionized calcium
I-PTH	intact parathyroid hormone
IRMA	immunoradiometric assay
NaCl	sodium chloride
N-PTH	amino-terminal parathyroid hormone
PTH	parathyroid hormone
PTH-Ca curve	parathyroid hormone-calcium curve
RIA	radioimmunoassay
tCa	total calcium
Vitamin D	calciferol
Vitamin D_2	ergocalciferol
Vitamin D_3	cholecalciferol
W-PTH	whole parathyroid hormone

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INTRODUCTION

The control of calcium, phosphorus, and magnesium is extremely important, not only for proper bone growth in young animals but also for normal cell stability, muscle contraction, and acid-base regulation. These minerals are regulated by three main body systems: gastrointestinal tract, kidneys, and parathyroid glands. Accordingly, any dysfunction in any one of these organ systems may result in severe disturbances of cellular function and growth.[46]

CALCIUM HOMEOSTASIS

Calcium is required for many vital intracellular and extracellular functions, as well as for skeletal support. It is the most abundant component of the skeleton and is an important cofactor for neural transmission, enzyme activity, blood coagulation, muscle contraction, hormone secretion, and other cellular functions.[91,92] Recent research in human beings indicates a link between calcium status and obesity and risk of diabetes mellitus among other diseases but links have not been made in domestic animals.[69]

Approximately 99% of body calcium resides in the skeleton and is stored as hydroxyapatite. Most skeletal calcium is poorly exchangeable, and less than 1% is considered readily available. Almost all of the non-skeletal calcium resides in the extracellular space, although small and biologically important quantities are found intracellularly.[92] Extracellular calcium exists in three fractions: ionized (iCa), complexed (bound to phosphate, bicarbonate, sulfate, citrate, and lactate), and protein bound. In clinically normal cats, protein-bound, complexed and iCa account for approximately 40%, 8%, and 52% of total serum calcium (tCa) concentration, respectively. Ionized calcium is the biologically active form of calcium and its homeostasis is important for many physiologic

functions.[100] Intracellular iCa is an important secondary messenger in the response to biochemical signals through the cell membrane.[92]

Regulation of serum calcium concentration is complex and requires the integrated actions of parathyroid hormone (PTH), vitamin D metabolites, calcitonin (CT), and iCa itself. Parathyroid hormone and calcitriol are the principal regulators of calcium homeostasis.[8,76,101,102] Parathyroid hormone is mainly responsible for the minute-to-minute control of serum iCa concentration, whereas calcitriol maintains day-to-day control of serum iCa concentration.[101] Calcium homeostasis in adult animals is regulated mainly by absorption from the gastrointestinal tract, excretion by the kidneys, and the rates of bone resorption and formation. In healthy animals, an equivalent amount of calcium is excreted primarily in the urine with small losses in sweat and intestinal secretions. The calcium released from bone by osteoclastic bone resorption and the calcium deposited in bone by bone formation are balanced in healthy adult animals. Intestinal calcium absorption is the principal determinant of the amount of calcium excreted in the urine in adult animals.[93]

Normal homeostatic response to hypocalcemia

Hypocalcemia elicits corrective responses that are mediated through the hormonal actions of PTH and calcitriol.[93] Acute effects occur in seconds to minutes, subacute effects occur over several hours, and chronic effects occur over days to weeks. A fall in iCa concentration is immediately sensed by the parathyroid glands, which respond with an increase in PTH secretion. Hypocalcemia decreases the proportion of PTH that is degraded in the parathyroid chief cells, making more PTH available for secretion. During increased PTH secretion, renal calcium absorption and phosphorus excretion are increased within minutes, whereas bone mobilization of calcium and phosphate occurs within 1 to 2 hours.[101]

After several hours of hypocalcemia, increased PTH secretion stimulates the synthesis and secretion of calcitriol. Increased intestinal transport of calcium and phosphorus into blood follows, providing an external source of calcium in addition to the internal mobilization from bone. Over days or weeks of hypocalcemia, further increases in PTH secretion are achieved largely by hypertrophy and hyperplasia of chief cells in the parathyroid gland.[94]

Normal homeostatic response to hypercalcemia

Most of the effects that occur during hypercalcemia are the opposite of those described above for hypocalcemia. A rise in iCa concentration causes a decrease in PTH secretion from the parathyroid glands.[25,93] Increased CT secretion is stimulated in an attempt to minimize the magnitude of hypercalcemia. In addition, hyperplasia of C cells in the thyroid gland results if the hypercalcemic stimulus is sustained, but this mechanism is ineffective for controlling hypercalcemia because of the transitory effect of CT on osteoclastic bone resorption.[28] Calcitriol synthesis is decreased both through direct inhibition by iCa and as a result of decreased stimulation because of decreased PTH concentration.[101]

In general, these hormonal responses are more effective in protecting against hypocalcemia than hypercalcemia. Perturbations in these mechanisms as exemplified by excessive increases in bone resorption, deficiencies or excesses of PTH or calcitriol, and defects in renal capacity to handle calcium and phosphate will lead to either hypo- or hypercalcemia.[76]

Calcium-Regulating Hormones

Parathyroid hormone

Parathyroid hormone is an 84-amino acid single-chain polypeptide that is synthesized and secreted by the chief cells of the parathyroid glands.[76] The amino acid sequence of PTH is known for the dog, cow, pig, rat, chicken, and human; and most mammals appear to have very similar amino-terminal portions of the molecule.[11,109]

Synthesis, secretion, and degradation of PTH by chief cells are closely related. After secretion, PTH has a short half-life (3 to 5 minutes) in serum; thus a steady rate of secretion is necessary to maintain serum PTH concentrations.[101]

The biological actions of PTH include (a) increase the blood calcium concentration; (b) increase calcium reabsorption and inhibition of phosphate reabsorption from the renal tubules, resulting in decreased calcium loss in the urine; (c) stimulation of osteoclastic bone resorption and the number of osteoclasts on bone surfaces and release of calcium and phosphate from bone; and (d) accelerate the formation of the principal active vitamin D metabolite (calcitriol) by the kidney, which increases intestinal absorption of calcium and phosphate.[8,76,101,102]

Parathyroid hormone metabolism is complex and produces several fragments of varying biological and immunological reactivity.[76] These fragments are themselves biologically active and their effects are mediated by a novel receptor.[79] Under normocalcemic conditions, circulating PTH is composed of 20% intact PTH (I-PTH)(1-84), and of 80% carboxyl-terminal (C) PTH fragments, considered until recently to be biologically inactive.[26] Carboxyl-terminal fragments missing the amino-terminal (N) structure of PTH(1-84) were identified first.[27] In renal failure, C-PTH fragments

accumulate because they are cleared mainly by the kidney in individuals with normal renal function; in this situation C-PTH fragments represent up to 95% of circulating PTH.[26,27] They are regulated by calcium slightly differently than PTH(1-84), occurring in a relatively smaller proportion relative to the latter in hypocalcemia but in a much larger proportion in hypercalcemia.[27] A second category of C-PTH fragments has a partially preserved N-structure. Studies in humans have also demonstrated the existence of smaller C-PTH fragments and, more recently, of larger C-PTH fragments with a partially preserved N-structure, called non-(1-84)PTH.[26,27] Carboxyl-terminal PTH fragments are acutely regulated in the circulation by iCa concentration.[27] They also exert similar hypocalcemic and antiresorptive effects. Overall, studies suggest that PTH(1-84) and C-PTH fragments are regulated differently to exert opposite biological effects on bone via two different receptors. This may serve to control bone turnover and calcium concentration more efficiently.[27,79]

The immunoheterogeneous nature of circulating PTH has given rise over the past 40 years to the ongoing replacement of PTH assays with increasing specificities.[26] Because of sequence homology of human and animal PTH, commercial assays developed for humans have been used successfully for some veterinary species.[5,33,35,36] A two-site immunoradiometric assay (IRMA) for intact human PTH which measures both the I-PTH (1-84) and the PTH (7-84) fragment has been validated in the dog and cat.[5,12,86,112] A new third-generation IRMA whole PTH (W-PTH) has been developed for use in humans that measures only the PTH (1-84) fragment. This novel assay could offer a better measure of W-PTH especially in patients with secondary hyperparathyroidism because the PTH (7-84) fragment is elevated in this disease.[42] High concentrations of C-PTH fragments, which occur in cats with chronic renal failure (CKD), may interfere with I-PTH immunoassays.[6] The W-PTH assay may also be of better diagnostic value in dogs than the I-PTH assay because PTH (7-84) fragments may be increased in dogs as compared with humans.[36] Whole

PTH (1-84) and I-PTH (1-84 and 7-84) have been measured in dogs[36] and horses.[35]

Circadian rhythms are known to influence hormonal secretion. Most hormones have been shown to change their plasma levels throughout the day due to cycles related to day-night, sleep-wake or activity-inactivity. Diurnal variations in plasma PTH have been demonstrated in humans[18,41] and dogs.[66] Except for minor diurnal variation, PTH secretion is relatively constant but may have a mild pulsatile pattern in response to minor fluctuations in the concentration of serum iCa.[16]

The PTH response to changes in iCa concentration can be evaluated through the study of the PTH-Ca curve.[15] The PTH-Ca curve has been widely studied in humans[39] and in many domestic animals.[1,9,15,33,36,71,110] The PTH-Ca curve is obtained by inducing changes in plasma calcium and recording values of plasma PTH at different calcium levels, in a range from hypo- to hypercalcemia. The PTH-Ca curve relationship is best represented by a sigmoidal function. Several parameters are derived from the study of the PTH-Ca curve:[37] *basal PTH* is the PTH concentration before initiating either hyper- or hypocalcemia; *maximal PTH* is the highest PTH concentration observed in response to hypocalcemia and additional reduction in plasma calcium does not further increase PTH value; *minimal PTH* is the lowest PTH concentration during suppression by hypercalcemia and a further increase in plasma calcium does not result in any additional reduction in PTH; *the ratio of basal to maximal PTH* is the basal PTH divided by the maximal PTH and this fraction is multiplied by 100 to obtain a percentage; *basal plasma calcium* is the plasma calcium concentration before initiating either hypo- or hypercalcemia; *maximal plasma calcium* is the plasma calcium concentration at which the PTH concentration was first observed to be maximal or within 10% of maximal PTH; *minimal plasma calcium* is the plasma calcium concentration at which the PTH concentration was first observed to be minimal or within 10% of

minimal PTH; and *the set point* defined as the calcium concentration corresponding to the midpoint PTH value between maximal PTH and minimal PTH concentrations,[15] or the calcium concentration corresponding to 50% of maximal PTH.[37] The set point is considered an indicator of the calcium concentration at which PTH secretion is stimulated.[37]

An interesting feature of this curve is the phenomenon of hysteresis. With respect to the PTH-Ca curve relationship, the term hysteresis indicates that for the same plasma calcium concentration, the plasma PTH is higher during induction of either hypo- or hypercalcemia than during the recovery periods.[1,24,110] Hysteresis has been demonstrated in humans,[24] horses,[110] and dogs.[1]

Vitamin D metabolites

Vitamin D (calciferol) is classified as a secosteroid hormone which plays a key role in calcium homeostasis through the classic pathway involving the skin, liver and, ultimately, renal production of calcitriol.[31] Vitamin D_2 (ergocalciferol) is produced by irradiation of plants with ultraviolet light, whereas vitamin D_3 (cholecalciferol) is produced endogenously in many species of animals.[23] The cholecalciferol metabolites 25-hydroxyvitamin D_3 (calcidiol), 1,25-dihydroxyvitamin D_3 (calcitriol), and 24,25-dihydroxyvitamin D_3 are the most important of at least 30 metabolites.[101] Calcidiol that is produced in liver is the major circulating form of vitamin D and is accepted as a measure of vitamin D status.[23] The circulating concentration of calcidiol reflects skin synthesis of cholecalciferol following ultraviolet irradiation, dietary intake of calciferols, and the degree of renal tubular reclamation of calcidiol-vitamin D binding protein following glomerular filtration. Calcitriol is the naturally occurring vitamin

D metabolite that has the greatest affinity for the vitamin D receptor in tissues.[29]

In humans, the requirement for vitamin D can be met by consumption of vitamin D_2 or D_3 or by synthesis of cholecalciferol in the skin. Cholecalciferol is synthesized in the skin from 7-dehydrocholesterol after exposure to ultraviolet light. Dogs and cats inefficiently photosynthesize vitamin D in their skin and consequently are dependent on vitamin D in their diet.[50,80] This may have lead to overzealous incorporation of vitamin D to commercial diets, hence the emerging reports of hypercalcemia in recent years due to an excessive vitamin D supplementation in diets for domestic animals.[72,117]

Calcidiol is converted by 1-α-hydroxylase in the kidney to the most active metabolite (calcitriol), or alternatively to 24,25-dihydroxyvitamin D_3 by 24-hydroxylase, present in many tissues.[102] The hydroxylation of calcidiol to calcitriol is stimulated by low plasma concentrations of calcium or phosphorus and by high PTH concentrations, as well as by CT, insulin-like growth factor-1 and/or growth hormone.[57] Hypocalcemia and CT directly stimulate 1-α-hydroxylation independent of PTH. Estrogens and testosterone may also increase calcitriol synthesis.[14]

Calcitriol is the only natural form of vitamin D with significant biologic activity. The main target organs for vitamin D are the intestine, bone, kidney, and parathyroid glands.[32] With PTH, the primary function of vitamin D is to maintain plasma calcium and phosphate concentrations within narrow physiological limits.[31] In the intestine, calcitriol stimulates the active absorption of calcium and phosphate; in the kidney stimulates the reabsorption of calcium and phosphate from the glomerular filtrate; in growing cartilage calcitriol and 24,25-dihydroxyvitamin D_3 influence the processes of cartilage maturation and calcification; and in calcified bone calcitriol acts as a regulator of bone resorption.[57]

Measurement of vitamin D metabolites is helpful in diagnosing disorders of calcium homeostasis. Calcidiol and calcitriol are the metabolites of clinical interest for detection of hypo- and hypervitaminosis D, and abnormalities of the renal hydroxylase system (eg, renal failure).[101] The metabolites of vitamin D are chemically identical in all species, thus receptor-binding assays or radioimmunoassay (RIA) developed for use in humans are satisfactory for the measurement of the same metabolites in animals.[50,54]

Calcitonin

Calcitonin is a 32-amino acid polypeptide hormone that is synthesized by C cells mainly located in the thyroid glands.[74] As in PTH synthesis, gene transcription results in a larger precursor molecule, preprocalcitonin, which is processed within the C cells.[102] Sequencing of CT has revealed homology ranging from 53% to 90% among the mammals (humans, rats, dogs, and horses) in which it has been studied.[49,74,111]

The iCa concentration is the most important regulator of CT secretion. An important role of CT is to limit the degree of postprandial hypercalcemia, thus reducing the absorption of calcium from the gastrointestinal tract. This effect, in concert with PTH, acts to maintain serum iCa concentration within a narrow range.[59,102] Calcitonin is a potent hypocalcemic hormone which acts predominantly on bone to inhibit osteoclastic bone resorption. There is also evidence for an action in the kidney to decrease tubular reabsorption of calcium, and in the brain and hypothalamus, where a number of actions have been reported.[28]

The role of CT in calcium homeostasis appears to decrease with increasing age, resulting in lower basal plasma CT concentrations with increasing age. Especially at an early age, but also in other conditions of

high calcium demand such as in pregnancy and lactation, the actions of CT are directed at avoiding hypercalcemia and consequently renal loss of calcium. Calcitonin enhances the deposition of calcium in bone and in the exchangeable calcium pool, and prevents excessive bone resorption by decreasing osteoclastic function. In addition, CT plays an important role in sustaining the concentration of calcitriol independent of PTH.[57,102] Calcitonin also stimulates renal 1-α-hydroxylase activity. The effect of CT on the calcitriol synthesis and its interaction with PTH are of considerable interest, since the stimulatory effect of PTH on calcitriol synthesis appears to be mediated by adenosine 3',5'-cyclic monophosphate (cAMP), and CT also stimulates cAMP production in the kidney.[13,106]

Despite these documented effects, the physiological role of CT has long been debated, with some suggesting that CT is an "enigmatic" or even "vestigial" hormone.[28] The effects of CT on normal calcium homeostasis are considered to be minor.[101] Recently, there have been significant advances using genetically modified mouse models to explore physiological roles for CT acting via its receptor, the CT receptor. Data from these studies allows the proposition that CT has important and related roles in (a) protecting the skeleton by regulating bone turnover and (b) maintaining calcium homeostasis.[28]

Calcitonin molecules are quite similar among different species.[59] Thus, heterologous assays (i.e. assays incorporating antibodies against the human CT molecule) can be used to reliably measure CT concentration in some other mammals. A specific assay for quantification of canine CT has been described[49] and recently, the usefulness of human CT assays for quantification of equine CT concentration has been demonstrated.[95]

Similarly to PTH, the relationship between CT and iCa can be studied through the CT-Ca curve, which describes the response to changes in iCa concentration. The CT-Ca curve has been studied in clinically normal and

uremic rats,[113,115,116] and in humans with CKD.[38] Among domestic animals, increases in circulating CT concentration secondary to acute increases in iCa concentration in dogs[49] and horses[97] have been reported. Several parameters derived from the PTH-Ca curve mentioned above can be extrapolated to the study of the CT-Ca curve: *basal CT* is the CT concentration before initiation of hypercalcemia; *maximal CT* is the highest CT concentration observed in response to hypercalcemia and additional increase in plasma calcium does not further increase CT value; *the ratio of basal to maximal CT* is the basal CT divided by the maximal CT and this fraction is multiplied by 100 to obtain a percentage; *the set point* is the plasma calcium concentration at which maximal CT secretion is reduced by 50%.

PHOSPHORUS HOMEOSTASIS

Phosphate in the mammalian body is present predominantly (85%) in the form of organic phosphate stored as hydroxyapatite in the mineralized matrix of bone with most of the remaining 14-15% occurring intracellulary in soft tissues. Of this remaining 15% of inorganic phosphate, 10% is protein bound and 5% is complexed with either calcium or magnesium.[23,93,104]

Phosphorus plays an essential role in cellular structure and function. A constituent of structural phospholipids in cell membranes and of hydroxyapatite in bone, phosphorus also is an integral component of nucleic acids and of phosphoproteins involved in mitochondrial oxidative phosphorylation. Energy for essential metabolic processes (eg, muscle contraction, neuronal impulse conduction, epithelial transport) is stored in high energy phosphate bonds of adenosine triphosphate. Phosphorus is

also important in the intermediary metabolism of protein, fat, and carbohydrate and as a component of glycogen.[30,93]

Phosphorus homeostasis requires a balance between dietary intake, exchange of phosphorus between extracellular and bone storage pools, and renal excretion. The physiological regulation of phosphorus is incompletely understood. It is interlinked with calcium homeostasis; both minerals are subject to control by the calciotropic hormones.[43]

Absorption of dietary phosphate is approximately 60% to 70% and occurs by active transport using a sodium-phosphate cotransporter and by passive diffusion.[93] Ingested organic phosphate is hydrolyzed in the gastrointestinal tract, liberating inorganic phosphate for absorption.[30] Calcitriol increases intestinal phosphate absorption. Low dietary content of phosphate results in increased renal production of calcitriol and increased renal phosphate reabsorption.[93] There is no evidence of a direct effect of PTH on intestinal phosphate absorption, and observed effects are probably mediated by the role of PTH in conversion of calcidiol to calcitriol.[30]

Renal excretion of phosphate is determined by the glomerular filtration rate and the maximum tubular reabsorption rate. High dietary intake of phosphorus decreases proximal tubular reabsorption, whereas low dietary intake can result in nearly 100% proximal tubular reabsorption of phosphate. Parathyroid hormone is the most important regulator of renal phosphate transport, and it decreases the maximum tubular reabsorption rate. The effects of calcitriol on renal phosphate transport are difficult to separate from the effects of calcitriol on PTH secretion and on phosphate transport in other organs (eg, intestine, bone).[30,93]

However PTH and calcitriol are not the only regulators of phosphate homeostasis, the existence of new players in phosphate homeostasis have

been recently identified and these discoveries have contributed to a better understanding the pathogenesis of some disorders of phosphate regulation.[22] A number of phosphorus-regulating hormones, termed the “phosphatonins” (fibroblast growth factor-23 (FGF-23), secreted frizzled-related protein 4, matrix extracellular phosphoglycoprotein, and FGF-7) have been proposed, and research is just beginning to be published on the role of these hormones in veterinary species. Fibroblast growth factor-23 is the most thoroughly studied of these hormones; in fact it is now considered to be a key regulator of plasma phosphorus concentration.[43] Initially, it was identified in human patients with a genetic phosphate wasting disorder. Fibroblast growth factor-23 is also present in healthy human subjects and is secreted primarily by osteocytes and osteoblasts, in response to hyperphosphatemia and increased plasma calcitriol concentrations. In patients with chronic kidney failure, FGF-23 levels increase as kidney functions deteriorate. Current investigations are focused to determine if the hormone actually participates in the pathophysiology of the deranged bone and mineral metabolism typical for these patients and, if so, whether it might serve as a therapeutic target.[3]

MAGNESIUM HOMEOSTASIS

Historically, magnesium has received very little attention in veterinary medicine as an electrolyte worthy of consideration.[10] Magnesium is an essential dietary element for animals. Approximately 55% of total body magnesium resides in the skeleton, and 45% is intracellular. Serum magnesium is comprised of three fractions (like calcium): free or ionized, protein bound, and complexed (bound to phosphate, bicarbonate, lactate, sulfate, acetate, and other compounds). Ionized magnesium accounts for 0.5-5% of total body magnesium and is the fraction that is biologically active.[99] Ionized magnesium is important in the support of enzyme activity

and is a cofactor for more than 300 enzymes. The action of magnesium extends to all major anabolic and catabolic processes. Thus, magnesium plays a pivotal role in muscle contraction; protein, fat, and carbohydrate metabolism; methyl group transfer; oxidative phosphorylation; functional properties and stabilization of membranes; cell division; and immune responses.[93]

Magnesium homeostasis is a result of the balance between intestinal absorption and renal excretion with additional regulation by the adrenals, thyroids, and parathyroid glands.[10,93] A number of hormones including PTH, CT, vitamin D, insulin, glucagon, antidiuretic hormone, aldosterone, and sex steroids have been reported to influence magnesium balance, notwithstanding the possibility that these may not be the primary regulators of magnesium homeostasis.[97]

The calciotropic hormones exert their influence on magnesium in the kidney, affecting magnesium reabsorption in the cortical part of the thick ascending limb of the loop of Henle and in the distal convoluted tubules by different cellular mechanisms. Parathyroid hormone stimulates magnesium reabsorption both in the loop of Henle and in the distal tubule. The PTH modulation of magnesium is mediated by activation of adenylate cyclase and production of cAMP.[97] Calcium may modulate the PTH action as in primary hyperparathyroidism when magnesium reabsorption is impaired due to a large renal calcium load resulting in hypermagnesuria. Also, magnesium levels may influence PTH secretion through a feedback system, thus chronic hypermagnesuria may suppress PTH secretion and cause disturbances in calcium homeostasis.[97,114,122] Vitamin D has been shown to enhance the intestinal absorption of magnesium through separate active transport mechanisms. However, this phenomenon may not play an important role in the overall magnesium homeostasis because of an increased urinary excretion.[97,122] Little is known about the effects of CT on magnesium. It has been reported that CT may stimulate renal

magnesium reabsorption in the rat. Calcitonin activates adenylate cyclase in different parts of the nephron that does PTH.[85]

Transcellular magnesium transport takes place mainly by intestinal absorption and renal excretion. Magnesium is absorbed as a freely diffusing ion by three mechanisms: (a) paracellular diffusion, (b) paracellular solvent drag, and (c) transcellular active transport.[93,97] Magnesium is absorbed mainly in the ileum and in the colon. The absorption is increased by calcitriol, but the mechanism of the transport is unknown and calcium and magnesium differ in the degree that absorption is stimulated. A common intestinal transport system for calcium and magnesium has been hypothesized.[10,93]

The three principal routes of magnesium excretion are the gastrointestinal tract, kidney, and mammary gland during lactation. The kidneys play a major role in regulating magnesium balance and serum concentration of magnesium by controlling tubular reabsorption.[10,93] Along the nephron, magnesium is reabsorbed in the proximal convoluted tubule (5-15%), the thick ascending limb of Henle (50-60%), and the distal convoluted tubule (10%).[114] Approximately 75% of the total plasma magnesium is filtered through the glomerular membrane. Under normal conditions only 3-5% of the filtered magnesium is excreted in the urine.[97] Magnesium appears in the urine when the filtered load exceeds the maximal tubular reabsorptive capacity. Urinary magnesium concentration can be used as an indicator of dietary supply but is not representative of current plasma magnesium concentration.[93]

INFLUENCE OF CALCULOLYTIC DIETS ON MINERAL METABOLISM

The prevalence of feline lower urinary tract disease (FLUTD), irrespective of cause, has been reported to be 1.5% to 8%. The majority of these cases (>60%) occurred in cats younger than 10 years of age and were identified as idiopathic cystitis. An estimated 10% to 20% of cats with FLUTD have urolithiasis or urethral plugs; struvite and calcium oxalate are found in more than 80% of these patients.[56]

One of the options in the treatment of FLUTD is based on dietary modification. Experimental and clinical investigations have confirmed the importance of dietary modifications in medical protocols designed to treat and prevent feline lower urinary tract signs. The main goals of dietary modification to prevent FLUTD are to: a) promote large dilute volumes of urine, b) decrease the relative supersaturation of urine for specific stone types, and c) promote healthy bacterial populations in the gastrointestinal and urogenital tracts, and d) increase the salt content to promote enhanced diuresis.[61]

The major strategies used by these diets are focused on the urine acidification (adjusting urine pH) and in the increase of salt content to enhance diuresis. The easiest way of reducing supersaturation and, indeed, one of the simplest and most effective treatments for all causes of FLUTD, is to increase urine volume and promote diuresis. There is a great deal of evidence in cats showing that low urine volume as well as high urine concentration are risk factors for urolith formation.[61]

Acidifying diets increase both urine concentration and fractional excretion (FE) of calcium, and magnesium restriction reduces the urine content of magnesium.[40] In addition, ingestion of acidifying diets restricted

in magnesium and marginal in potassium content was reported to cause potassium depletion, hypokalemia, and CKD. Based on experimental evidence, bone homeostasis also might be affected by acidifying diets.[17] Increased urine calcium excretion and bone demineralization were found in some adult cats that were fed diets containing 1.5% ammonium chloride.[20] It has been reported that chronic ingestion of ammonium chloride produces metabolic acidosis and alterations in calcium metabolism. During metabolic acidosis, the responses of kidney, intestine, and bone may be mediated by changes in calcitriol and PTH. Some data indicate that the effects on calcium metabolism are independent of PTH and dietary levels of calcium. There is a paucity of information regarding the metabolism of other minerals in metabolic acidosis, but phosphorus, magnesium, potassium, chloride, and sodium metabolism may be variably affected. Studies have demonstrated that chronic metabolic acidosis may lead paradoxically to potassium depletion with the development of hypokalemia. In addition to changes in the metabolism of each of these minerals, combined alterations in calcium, potassium, and magnesium metabolism can be seen in metabolic acidosis, due to their physiological interrelationships. Renal handling of sodium and chloride may also be affected by metabolic acidosis secondary to decreased filtered bicarbonate and reduced bicarbonate reabsorption in the renal proximal tubule.[20]

Moreover, increasing salt intake to promote diuresis has been suggested in the management of FLUTD. Increasing urine volume, urine dilution, or both can be reliably achieved in healthy cats by feeding a dry diet with an increased content of salt. One study reported that serum creatinine, urea, and phosphorus concentrations increased when cats were fed a high salt diet[62] whereas in other studies the markers of kidney function were unaffected by a high dietary salt intake.[89,120]

Historically, there has been controversy about the use of sodium chloride to stimulate thirst and diuresis, as it could also potentially affect

urinary calcium excretion, blood pressure and renal disease.[81] However, recent studies in cats have refuted this theory, and support the use of moderate increases in sodium to help maintain urinary tract health. On the other hand, while sodium chloride (NaCl) has been reported to increase urinary calcium excretion[62] other authors have not found effect on salt on calcium or magnesium excretion by urine.[120] Recent work supports a link between salt consumption and renal handling of calcium and magnesium, which is reflected by changes in PTH.[64] In humans, high salt intake has been associated with increased urinary calcium excretion, and similar observations were initially made in dogs.[67] This led to the assumption that salt-enriched diets could promote calcium oxalate urolithiasis and that diets for management of FLUTD should thus be salt-restricted.[81]

Aims and Scope

The present work aims to study mineral metabolism in cats, including the relationship between calcium metabolism and major hormones involved in it, the main changes in the parameters of feline mineral metabolism during the juvenile stage and, finally, the study of mineral metabolism in cats fed with calculolytic diets.

Thus, the Doctoral Thesis has the following objectives:

1. **To validate commercially available human I-PTH and W-PTH assays for measurement of feline PTH**

Since there are no specific assays for the quantification of feline PTH, the ability to use assays designed for the quantification of human PTH and to employ them for measurement in other species is an aspect which has a great relevance in the field of veterinary medicine. In previous investigations our research group conducted similar studies in other animal species.[33,35] Focusing on the feline species, there are reports in the literature about validated assays for quantification of I-PTH in cats, but currently these assays are no longer available.[5,12,86] Moreover, the third-generation W-PTH assay that is replacing the gold standard position because of its specificity and apparently clinical utilities has not been evaluated in cats.

Our hypothesis is linked with the fact that commercial assays developed for humans have been used successfully for some veterinary species, due to sequence homology of human and animal PTH. Therefore, we hypothesize that measurement in feline samples with a human PTH assay should be suitable for the quantification of this hormone in cats.

2. To characterize the dynamics of PTH secretion in response to changes in extracellular calcium in healthy cats

The study of dynamics of PTH secretion through the PTH-Ca curve provides important insights of the regulatory mechanisms involved in calcium homeostasis and represents the basis to understand the pathophysiology of endocrine disorders of calcium metabolism. Our research group has extensive experience in the study of the PTH-Ca curve in several species.[1,9,33,36]

Our hypothesis is that changes in plasma calcium concentration, in a range from hypo- to hypercalcemia, will induce changes in plasma PTH levels which allow characterization of the feline PTH-Ca curve.

3. To describe changes in the main parameters involved in mineral metabolism (calcium, phosphorus, magnesium, PTH, calcitriol, and calcidiol) during the growing process in healthy cats aged 3 to 15 months

In cats, knowledge about blood parameters related to mineral metabolism is scanty and there are only a few reports about changes in plasma minerals with growth.[63,65] Moreover, apart from scattered measurements obtained in healthy young animals to compare with clinical cases, no information is available on changes in calciotropic hormones in growing cats. Processes affecting young animals such as rickets or nutritional secondary hyperparathyroidism are described in the literature.[44,45,53,68,82,103,107,108] In these cases the cornerstone of the diagnosis is focused on the quantification of the main parameters involved in mineral metabolism. Thus, reliable reference values for young patients are needed for accurate diagnosis.

We hypothesize that mineral metabolism in growing animals is subjected to changes derived from the different rates of bone growth along time and these changes are reflected in the blood concentrations of minerals and hormones that participate in their homeostasis. Thus, blood parameters of mineral metabolism should be different in the young growing animal and in the adult.

4. To validate a commercially available human CT assay for quantification of feline CT and to provide normal values in healthy cats

Information about CT in domestic animals is fragmentary. A specific assay for quantification of canine CT has been described[49] and measurement of plasma CT concentrations in horses has been recently reported,[95] but to our knowledge no similar data are available for cats.

Our hypothesis is that assays that quantify human CT can be used for measurement of feline CT.

5. To characterize the dynamics of CT secretion in response to experimentally induced hypercalcemia and the related changes in extracellular iCa concentration in healthy cats

Although CT has always been considered a hormone with a secondary role in mineral metabolism, the study of dynamics of CT secretion to changes in calcium concentration would be interesting. Abnormalities of calcium metabolism such as hypercalcemia of malignancy, hypo- and hypercalcemia associated with renal failure and nutritional disorders are common in cats.[7,98] Moreover, cats can develop derangements of calcium

metabolism such as idiopathic hypercalcemia, which has unknown etiopathogenesis.[73]

We hypothesized that changes in plasma calcium concentration, in a range from hypo- to hypercalcemia, will induce changes in plasma CT levels which allow characterization of the feline CT-Ca curve.

6. To evaluate the influence of two calculolytic diets on parameters of mineral metabolism in cats

Experimental and clinical investigations have confirmed the importance of dietary modifications in medical protocols designed to treat and prevent feline lower urinary tract signs. Thus, nutritional management with calculolytic diets is quite effective in preventing and treating struvite uroliths in cats.[61] These diets are formulated to modify urine by reducing pH, specific gravity and mineral content.[61] In addition, because their composition, these diets promote a higher increase in water consumption.[62,120] Acidosis is known to have an influence in mineral metabolism by increasing PTH secretion, promoting bone resorption, decreasing vitamin D and altering urinary handling of minerals. Lowering urine pH may influence the urinary excretion of minerals, like calcium and magnesium.[120]

Our hypothesis is that feeding calculolytic diets would promote excessive urinary waste of minerals and, as a consequence, would modify blood parameters of mineral metabolism.

SCIENTIFIC ARTICLES

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Feline parathyroid hormone: validation of hormonal assays and dynamics of secretion

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Abstract

Validated assays for quantification of intact parathyroid hormone (I-PTH) are no longer available. Moreover, the third-generation PTH assay that only detects the whole PTH molecule (W-PTH) has never been tested in cats. The work presented here is aimed to validate a commercially available assay for measurement of I-PTH and W-PTH in cats and to study the dynamics of PTH secretion in healthy cats. Our results show that both assays are reliable for the measurement of feline PTH. In healthy adult cats W-PTH concentration (15.1 ± 1.6 pg/mL) was greater ($P < 0.001$) than I-PTH concentration (9.1 ± 0.7 pg/mL). The dynamics of PTH secretion in response to changes in extracellular calcium (Ca^{2+}) were investigated in 13 cats by studying PTH-Ca^{2+} curves. PTH-Ca^{2+} curves were obtained by intravenous infusion of disodium ethylenediaminetetraacetic acid and $CaCl_2$. PTH was measured using both I-PTH and W-PTH assays. During hypocalcemia a sigmoidal curve that was similar when measured with I-PTH or W-PTH was obtained. The maximal PTH concentration in response to hypocalcemia was greater with W-PTH (179.6 ± 41.9 pg/mL) than with I-PTH (67.6 ± 10.5 pg/mL; $P = 0.01$). However, hypercalcemia resulted in an equivalent PTH inhibition, with both assays yielding PTH concentrations as follows: W-PTH = 4.0 ± 0.4 pg/mL and I-PTH = 4.9 ± 0.3 pg/mL (NS). Parameters of the feline PTH-Ca^{2+} curve are similar to what has been previously reported in dogs.
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Keywords: Cat; Intact PTH; PTH–calcium curve; Whole PTH

1. Introduction

Parathyroid hormone (PTH) plays a major role in the regulation of mineral metabolism, particularly in the control of extracellular calcium concentrations. Basically, PTH is a hypercalcemic hormone secreted by the chief cells in the parathyroid glands in response to hypocalcemia [1,2]. Parathyroid hormone has been studied in domestic animals, especially in dogs and horses [3–5]; however, knowledge in feline medicine is incomplete.

As in other species, measurement of PTH is complicated by the existence of a number of circulating PTH fragments that can potentially interfere with hormonal quantification [6]. Thus, first-generation PTH assays, which bind to PTH fragments, have limited usefulness for PTH quantification. Until recently, the second-generation two-site immunoradiometric assay (IRMA), also known as intact PTH assay (I-PTH), has been considered the gold standard for PTH measure-

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ment in humans [7,8] and has been validated for use in cats [9]. However, the assay that was validated for measurement of intact PTH in cats (Allegro Intact Parathyroid Hormone Immunoassay, Nichols Institute Diagnostics, San Juan Capistrano, CA) is no longer available and, to our knowledge, no information exists regarding the utility of other equivalent human assays for measurement of feline I-PTH. Moreover, the third-generation whole PTH (W-PTH; cyclase activating PTH) assay that is replacing the gold standard position because of its specificity and apparently superior clinical utilities [10] has not been evaluated in cats.

In most species the relationship between PTH and blood ionized calcium (Ca^{2+}) is best represented by a sigmoidal curve: the PTH-Ca^{2+} curve [11]. The PTH-Ca^{2+} curve has been extensively studied in humans [12] and in many domestic animals, including dogs [13], horses [3,14], cows [15], and rabbits [16]. However, other than the well-known fact that PTH increases during hypocalcemia and decreases during hypercalcemia, little information on the dynamics of PTH secretion is available in cats. Knowledge of the PTH-Ca^{2+} curve provides important insights of the regulatory mechanisms involved in calcium homeostasis and represents the basis to understand the pathophysiology of endocrine disorders of calcium metabolism.

The work reported here is aimed to validate a commercially available human I-PTH and W-PTH assay for measurement of feline PTH and to characterize the dynamics of PTH secretion in response to changes in extracellular calcium in healthy cats.

2. Materials and methods

2.1. Assay validation

The PTH (I-PTH and W-PTH) assays were validated by assessment of linearity, sensitivity, accuracy, precision, and reproducibility.

2.1.1. Linearity

Dilutional parallelism was used to assess the linearity of the assays. Feline plasma with high PTH concentration was serially diluted (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256) with the zero standard of each assay. Duplicate measurements were obtained in each dilution. The values of PTH were plotted against their respective dilution factors and the measured concentration corrected by the dilution factor (the apparent recovery) was compared with the undiluted value.

2.1.2. Sensitivity

Sensitivity of the assays was also calculated from the dilution curves. Sensitivity was defined as the point at which the lower 95% confidence limit of the dilution intercepts the x-axis [3].

2.1.3. Accuracy

Accuracy of the assays was determined by spiking recovery [10]. Plasma samples with three concentrations of PTH (I-PTH = 2.7, 6.3, and 26.7 pg/mL, W-PTH = 4.0, 8.9, and 36 pg/mL) were spiked with a plasma sample with greater PTH concentration (I-PTH = 39.5 pg/mL, W-PTH = 62.5 pg/mL). Spiking was performed in two experiments: in the first experiment the samples were mixed with an equal volume of plasma with greater PTH concentration (1:1 dilution) and in the second experiment the samples were mixed with three times the volume of plasma with high PTH concentration (1:3 dilution). The apparent recovery was calculated by comparing measured values with values extrapolated from the dilutions.

2.1.4. Precision

Intra-assay variability, which was used to test precision, was determined for each assay in plasma samples containing normal and high PTH concentrations. Samples with normal PTH concentrations were obtained from healthy cats; samples with high PTH concentrations were obtained from healthy cats with experimentally induced hypocalcemia. For calculation of the intra-assay variability a sample was measured 10 times, in nonconsecutive position, using the same assay.

2.1.5. Reproducibility

Reproducibility was tested by calculation of interassay variability, which was determined by assaying a sample in eight different assays.

The coefficients of intra- and interassay variability were calculated as the standard deviation expressed as a percentage of the mean.

2.2. Normal range of I-PTH and W-PTH in healthy cats

Heparinized plasma samples (n = 52) were obtained from adult healthy cats on a standard chow diet with normal mineral content (Ca = 1.1%, P = 1%, vit D = 1,500 IU/kg). Cats were of European short-hair breed and belonged to the Animal House Facility of the University of Cordoba. Cats were not neutered, with an age (mean ± standard error) of 16.9 ± 0.2 mo, and there was an equivalent distribution between sexes. Cats were considered healthy on the basis of a

normal physical examination, normal hemogram, and normal biochemical profile, which included the following parameters: calcium, phosphorus, magnesium, sodium, potassium, chloride, bicarbonate, albumin, and creatinine.

2.3. Dynamics of PTH secretion in response to hypo- and hypercalcemia

The PTH-Ca^{2+} curves were obtained in 13 European short-hair cats of both sexes (6 males, 7 females), aged 16 to 18 mo and weighing 3.8 ± 0.3 kg. Cats were kept in a cattery belonging to the Animal House Facility of the University of Cordoba and fed a diet with normal mineral content (Ca = 1.1%, P = 1%, vit D = 1,500 IU/kg). The animals had been socialized by frequent human contact to prevent stress associated with handling. All experimental procedures were approved by the ethics committee of the University of Cordoba.

PTH-Ca^{2+} curves were obtained by intravenous infusion of disodium ethylenediaminetetraacetic acid (EDTA) and $CaCl_2$. Cats were anesthetized by a combination of 15 mg kg^{-1} ketamine and 0.4 mg kg^{-1} midazolam. A jugular vein and the contralateral cephalic vein were cannulated with 18- and 20-G catheters, respectively. The cephalic venous port was used for EDTA and $CaCl_2$ infusion and the jugular venous side was used for blood sampling. The protocols for induction of hyper- and hypocalcemia were as follows:

- *Hypercalcemia.* Hypercalcemia was achieved by intravenous infusion of $CaCl_2$, which was started at a rate of 0.27 mEq of calcium/kg/h. $CaCl_2$ infusion was increased every 5 min up to a final rate of 0.55 mEq of calcium/kg/h at 50 min.
- *Hypocalcemia.* Hypocalcemia was induced by a Na_2EDTA infusion, which was initiated at a rate of 60 mg/kg/h. To achieve a linear decrease in Ca^{2+}, the rate of the EDTA infusion was progressively increased every 5 min, up to 132 mg/kg/h at the end of the experiment (50 min).

The order of the protocols (hyper- vs hypocalcemia) was randomly allocated. In six cats hypercalcemia was induced first and in the other seven animals hypocalcemia was induced first. An interval of 1 wk was allowed between both experiments.

Three blood samples were obtained from each animal as baseline; thereafter, blood samples were collected every 5 min until the end of the experiments. In the hypocalcemic studies additional blood samples were obtained over 1 h to evaluate recovery from hypocalcemia.

Individual PTH-Ca^{2+} curves were constructed by adjusting the PTH and Ca^{2+} values of every cat to a sigmoidal equation. The PTH concentrations at standardized Ca^{2+} concentrations (from Ca^{2+} = 1.20 mM to Ca^{2+} = 0.75 mM, with an interval of 0.05 mM) were extrapolated from these individual curves. Mean PTH values at standardized Ca^{2+} concentrations were used to obtain the PTH-Ca^{2+} curve for each group.

The following parameters were derived from the PTH-Ca^{2+} curves [17]:

1. Basal PTH (PTH_b) is the PTH concentration before initiating either hyper- or hypocalcemia.
2. Maximal PTH (PTH_{max}) is the highest PTH concentration observed in response to hypocalcemia and additional reduction in plasma calcium does not further increase PTH value.
3. Minimal PTH (PTH_{min}) is the lowest PTH concentration during suppression by hypercalcemia and a further increase in plasma calcium does not result in any additional reduction in PTH.
4. The ratio of basal to maximal PTH ($PTH_{b/max}$) is the basal PTH divided by the maximal PTH and this fraction is multiplied by 100 to obtain a percentage.
5. The basal plasma Ca^{2+} (Ca_b) is the plasma Ca^{2+} concentration before initiating either hyper- or hypocalcemia.
6. The plasma Ca^{2+} at maximal PTH (Ca_{max}) is the plasma Ca^{2+} concentration at which the PTH concentration was first observed to be maximal or within 10% of maximal PTH.
7. The plasma Ca^{2+} at minimal PTH (Ca_{min}) is the plasma Ca^{2+} concentration at which the PTH concentration was first observed to be minimal or within 10% of minimal PTH.
8. The set point (SP) of Ca^{2+} has been calculated in two different ways: (a) as the plasma Ca^{2+} concentration at which maximal PTH secretion is reduced by 50% (SP1) and (b) as the plasma Ca^{2+} concentration at which the difference between PTH_{max} minus PTH_{min} is reduced by 50% (SP2).

2.4. Laboratory measurements

Blood Ca^{2+} was measured immediately after collection using selective electrodes (Bayer Diagnostics, Barcelona, Spain); then, samples were centrifuged and plasma was frozen at −20°C. PTH was measured in plasma samples within 2 mo of collection using IRMA. PTH was measured in all samples with the Duo PTH kit (Scantibodies Laboratory, Inc., Santee, CA). This kit

has been designed for the quantitative determination of human W-PTH and I-PTH. The kit contains two IRMA. Both assays share a polyclonal antibody (anti-PTH 39–84) coated onto the surface of polystyrene beads as a solid phase. The IRMA for W-PTH utilizes a tracer antibody directed against the most N-terminal PTH (1–4) region. The use of this antibody guarantees that only biologically active PTH is detected. The IRMA for I-PTH uses a specific polyclonal antibody directed against PTH (7–34) as a tracer. With this antibody, both W-PTH (1–84) and N-truncated PTH fragments (7–84) are detected.

2.5. Statistical analysis

Statistical analysis was performed using the computer package SPSS for Windows 15.0 (SPSS, Inc., Chicago, IL). A Kolmogorov-Smirnov test was carried out to test for normality. All data sets passed normality testing: Ca^{2+} (P = 0.179), I-PTH (P = 0.393), and W-PTH (P = 0.366). I-PTH and W-PTH changes along the range of Ca^{2+} in the PTH-Ca^{2+} curves were studied by unpaired Student *t* tests—the PTH values at each Ca^{2+} concentration were compared with the PTH concentration at baseline calcium. Comparison between PTH concentrations at the same Ca^{2+} concentration, during induction of and recovery from hypocalcemia, were carried out with the paired Student *t* test. *P* values less than 0.05 were considered significant. Values are given as the mean ± standard error.

3. Results

3.1. Assay validation

3.1.1. Linearity

Samples with high PTH concentration diluted in a predictable manner when measured with both W-PTH and I-PTH assays (Fig. 1). The agreement between measured and expected I-PTH values was excellent (Table 1). Some reduction in recovery (measured values lower than expected) was noted with the W-PTH assay. The percentage of recovery of W-PTH decreased along the dilution curve (Table 1).

3.1.2. Sensitivity

The sensitivity of the PTH assays was 2 pg/mL for I-PTH and 3 pg/mL for W-PTH. Thus, the working range of the assays would be 2 to 2,350 pg/mL for I-PTH and 3 to 2,180 pg/mL for W-PTH (the upper range has been obtained from the calibration curve of the assays).

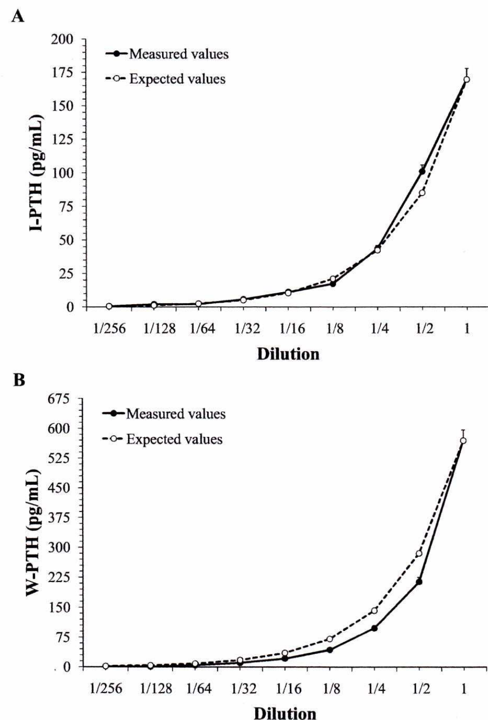

Fig. 1. Dilutional parallelism of parathyroid hormone (PTH). (A) Dilution of a representative feline plasma sample with high PTH concentration measured with the intact PTH (I-PTH) assay. Dilution was made with the zero standard of the assay. (B) Dilution of a representative feline plasma with high PTH concentration measured with the whole PTH (W-PTH) assay. Dilution was made with the zero standard of the assay. Measured values are represented by a continuous line and expected values (initial hormone concentration corrected for the dilution factor) are represented by a broken line.

3.1.3. Accuracy

The percentages in sample spiking recovery are shown in Table 1. In samples with final I-PTH concentration of 31.1 ± 4.2 pg/mL (range 18.1 to 47.2 pg/mL), spiking recovery was 104.7 ± 6.8% (range 85.6% to 130.2%). In samples with a final W-PTH concentration of 44.9 ± 5.0 pg/mL (range 32.4 to 62.4 pg/mL), the spiking recovery for was 102.3 ± 4.3% (range 90.8% to 116.5%).

3.1.4. Precision

In samples with normal PTH concentration (I-PTH = 6.7 ± 0.2 pg/mL, W-PTH = 9.9 ± 0.35 pg/mL), intra-assay variability was 9.5% for the I-PTH

Table 1
Dilutional parallelism and spiking recovery of the intact parathyroid hormone (I-PTH) and whole PTH (W-PTH) assays.

	I-PTH assay			W-PTH assay		
	Measured (pg/mL)	Expected (pg/mL)	Recovery (%)	Measured (pg/mL)	Expected (pg/mL)	Recovery (%)
Dilution						
1/2	100.9	85	118.7	213.5	284.8	75
1/4	43.8	42.5	103.1	98.3	142.4	69.1
1/8	17.4	21.3	82.1	43.5	71.2	61
1/16	11.4	10.6	106.9	21.6	35.6	60.7
1/32	5.9	5.3	111.3	11	17.8	61.6
1/64	2.4	2.7	90.2	4.9	8.9	54.6
1/128	2.2	1.3	163.9	1.4	4.5	31.5
1/256	0.5	0.7	74.2	1.4	2.2	61.3
Spiking						
S1 (1:1)	18.1	21.1	85.6	32.4	35.7	90.8
S2 (1:1)	22.1	22.9	96.8	32.8	33.2	98.8
S3 (1:1)	30.2	33.1	91.4	40.8	39.2	104.1
S1 (1:3)	33.4	30.3	110.4	45.1	49.1	91.9
S2 (1:3)	35.5	31.2	113.8	55.7	47.8	116.5
S3 (1:3)	47.2	36.3	130.2	62.4	55.8	111.7

Spiking: (1:1) samples were mixed with an equal volume of plasma with greater PTH concentration; (1:3) samples were mixed with three times the volume of plasma with high PTH concentration (see text for details). The apparent recovery was calculated by comparing measured values with values extrapolated from the dilutions.

assay and 11.4% for the W-PTH assay. Intra-assay variability in samples with high PTH concentration (I-PTH = 173.5 ± 2.9 pg/mL, W-PTH = 580.1 ± 11.9 pg/mL) was 5.2% and 6.5% for the I-PTH and W-PTH assays, respectively.

3.1.5. Reproducibility

When calculating interassay reproducibility, values of 9.8% (normal PTH = 12.6 ± 0.44 pg/mL) and 11.1% (high PTH = 187.4 ± 7.4 pg/mL) were obtained with the I-PTH assay. The W-PTH assay had an interassay variability of 13.1% and 13.4% for samples with normal (20.8 ± 0.96 pg/mL) and high (525.6 ± 24.9 pg/mL) PTH, respectively.

3.2. Normal range of I-PTH and W-PTH in healthy cats

A scatter plot depicting PTH concentrations in healthy cats is shown in Fig. 2. Measurement of PTH concentration in samples from healthy adult cats using the I-PTH assay yielded a value of 9.1 ± 0.7 pg/mL, with a range of 1.6 to 23.7 pg/mL and a central 95th percentile from 2 to 19 pg/mL. When using the W-PTH assay, PTH values were 15.1 ± 1.6 pg/mL (range, 1.4 to 51.5 pg/mL; central 95th percentile, 3 to 37 pg/mL).

3.3. Dynamics of PTH secretion in response to hypo- and hypercalcemia

Fig. 3 shows the hypocalcemic component of the PTH-Ca^{2+} curve measured with the I-PTH and W-PTH assays. At baseline Ca^{2+} (Ca_b = 1.20 ± 0.01 mM) cats had an I-PTH concentration of 11.6 ± 2.4 pg/mL and a W-PTH concentration of 18.6 ± 5.5 pg/mL. Induction of hypocalcemia resulted in an increase in PTH concentration that was significant below Ca^{2+} = 1.15 mM and reached a maximum of 67.6 ± 10.5 pg/mL (I-PTH) and 179.6 ± 41.9 (W-PTH) at Ca^{2+} concentrations (Ca_{max}) of 0.82 ± 0.02 mM. The SP of the PTH-Ca curve was almost identical when calculated as SP1 or SP2, with values of 1.10 ± 0.03 (SP1) and 1.11 ± 0.03 mM (SP2) for I-PTH and 1.06 ± 0.03 mM (both SP1 and SP2) for W-PTH (Table 2).

Hysteresis of the hypocalcemic part of the PTH-Ca^{2+} curve was observed both with I-PTH and with W-PTH measurements. For a given calcium concentration, PTH values during recovery from hypocalcemia were lower than the corresponding PTH values measured during induction of hypocalcemia. Although hysteresis was consistent along the whole PTH-Ca^{2+} curve, significant differences between induction of and recovery from hypocalcemia were only observed at Ca^{2+} values ranging from 1 to 1.15 mM (Fig. 3).

Induction of hypercalcemia resulted in a decrease in PTH concentrations that was already significant at Ca^{2+} = 1.25 mM and reached minimum values at Ca^{2+} (Ca_{min}) = 1.59 ± 0.03 mM. Minimal PTH concentrations were similar for I-PTH = 4.9 ± 0.3 pg/mL and W-PTH = 4.0 ± 0.4 pg/mL (Fig. 4).

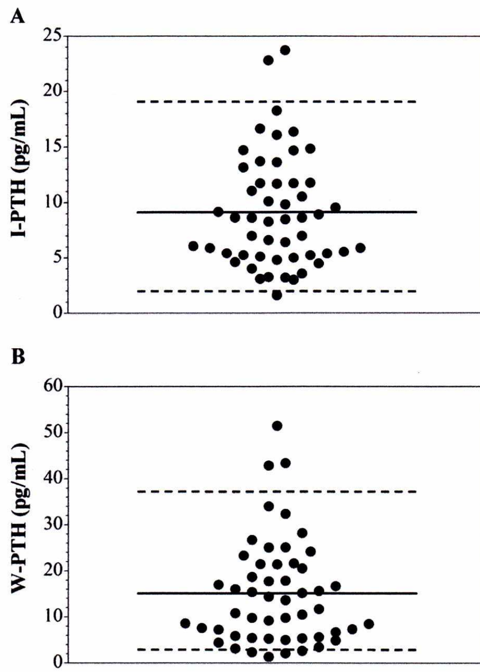

Fig. 2. Scatter plot of parathyroid homone (PTH) values in a population of healthy cats. (A) Intact PTH (I-PTH). (B) Whole PTH (W-PTH). Circles are individual PTH values, the horizontal line is the arithmetic mean, and the broken lines represent the central 95th percentile. Because the statistically calculated low 95th percentile was lower than the sensitivity of the assays, the lower percentile has been set at the sensitivity limit for each assay.

4. Discussion

In cats, as well as in most domestic animals, endocrine studies are often complicated by the lack of assays using specific antibodies. Although the above may be regarded as an important problem for quantification of calciotropic hormones in animals, it should be noted that the PTH molecule is similar in different species [18, 19]. Thus, heterologous assays (ie assays incorporating antibodies against the human PTH molecule) can be used to reliably measure PTH in some mammals. Human PTH assays have been successfully validated for measurement of canine [20], feline [9], and equine [3] PTH.

Although a two-site human IRMA has already been validated for measurement of feline PTH [9], this assay is no longer available. In the present study, a commercially available assay for measurement of I-PTH and a third-generation IRMA that detects exclusively the biologically active whole parathyroid hormone or cyclase activating PTH (W-PTH) have been validated for measurement of feline PTH.

The precision (9.8% to 11.1%) and sensitivity (2 pg/mL) of the I-PTH assay validated in this study has been found to be slightly better than what had been previously reported by Barber et al (precision, 12.7%; sensitivity, 3.9 pg/mL [9]) using the Allegro Intact PTH assay. When compared with the I-PTH assay, the W-PTH assay has similar intra-assay precision. However, the interassay coefficients of variability were somewhat

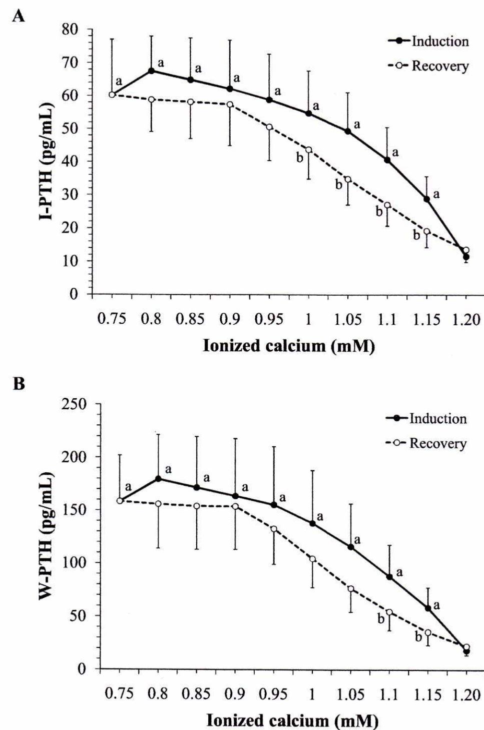

Fig. 3. (A) The hypocalcemic part of the parathyroid hormone-extracellular calcium (PTH-Ca^{2+}) curve measured with the intact PTH (I-PTH) assay. (B) The hypocalcemic part of the PTH-Ca^{2+} curve measured with the whole PTH (W-PTH) assay. Both curves were obtained after inducing hypocalcemia to 13 cats (see Materials and Methods for details). Solid circles represent PTH values obtained during induction of hypocalcemia and open circles represent PTH values obtained during recovery from hypocalcemia. $^{a}P < 0.05$ vs PTH values at baseline calcium. $^{b}P < 0.05$ vs the PTH value obtained at the same calcium concentration during induction of hypocalcemia. Values are means ± standard error.

Table 2
Variables derived from the parathyroid hormone-extracellular calcium (PTH-Ca^{2+}) curve obtained in 13 healthy cats (see text for details).

Assay type	Assay variables					
	PTH_b (pg/mL)	PTH_{max} (pg/mL)	PTH_{min} (pg/mL)	$PTH_{b/max}$ (%)	SP1 (mM)	SP2 (mM)
I-PTH (n = 13)	11.60 ± 2.37	67.56 ± 10.45	4.93 ± 0.30	16.44 ± 3.57	1.10 ± 0.03	1.11 ± 0.03
W-PTH (n = 13)	18.62 ± 5.52	179.63 ± 41.88[a]	4.02 ± 0.36	13.57 ± 3.70	1.06 ± 0.03	1.06 ± 0.03
W-PTH/I-PTH (n = 13)	1.60 ± 0.15	2.46 ± 0.20[b]	0.85 ± 0.09[b,c]	—	—	—

PTH_b (basal PTH), PTH concentration before initiating either hyper- or hypocalcemia; PTH_{max} (maximal PTH), highest PTH concentration observed in response to hypocalcemia and additional reduction in plasma calcium does not further increase PTH value; PTH_{min} (minimal PTH), lowest PTH concentration during suppression by hypercalcemia and a further increase in plasma calcium does not result in any additional reduction in PTH; $PTH_{b/max}$ (ratio of basal to maximal PTH), basal PTH divided by the maximal PTH and this fraction is multiplied by 100 to obtain a percentage; set point 1 (SP1), plasma calcium concentration at which maximal PTH secretion is reduced by 50%; set point 2 (SP2), plasma calcium concentration at which the difference between PTH_{max} minus PTH_{min} is reduced by 50%. Comparison between the I-PTH and W-PTH assays:
[a] $P < 0.05$ vs I-PTH. Comparison of the W-PTH/I-PTH ratio at different PTH concentrations:
[b] $P < 0.05$ vs PTH_b; [c] $P < 0.05$ vs PTH_{max}.

greater when measuring W-PTH. This is in contrast to the data of Estepa et al [3], who found better reproducibility for W-PTH than for I-PTH in equine plasma. It is unlikely that species differences account for this discrepancy. Variability in the assay production and/or sample handling seems a more likely explanation. Both assays are sensitive and capable of detecting PTH values below the minimum PTH concentration that is registered during hypercalcemic inhibition of PTH secretion.

In normal cats, the I-PTH assay yielded values that were similar, 9.1 ± 0.7 pg/mL, to what has been previously reported in the literature using the assay validated by Barber et al, 10.9 ± 5.3 pg/mL (means ± standard deviation) [9]. Surprisingly, greater PTH values were registered when measuring PTH with the W-PTH assay than when using the I-PTH assay. This finding is unusual because I-PTH is supposed to measure all the W-PTH peptide (PTH 1–84) plus a number of truncated N-terminal fragments (PTH 7–84); however, it was not completely unexpected because the same phenomenon has been previously reported by our laboratory when measuring equine PTH [4].

The greater W-PTH than I-PTH values may be related to different affinity of the W-PTH and I-PTH tracer antibody against feline PTH. Thus, the tracer antibody against human 7–34 PTH, used by the I-PTH assay, would have lower affinity for the feline PTH molecule than the tracer antibody against 1–4 PTH, used in the W-PTH assay. If this were the case, the I-PTH assay would be detecting both 1–84 PTH and N-terminal truncated fragments, but its ability to bind the 7–34 region would be inferior to the binding capacity of the antibody against the 1–4 region and, consequently, the PTH concentration would be lower when measured with I-PTH than when measured with W-PTH. This hypothesis seems reasonable because the part of the PTH molecule that shows less interspecies variation is the amino-terminal end [21]. In addition, feline PTH has been reported to be similar to human PTH in the amino-terminal portion [18]; therefore, heterologous antibodies should be more likely to bind the most amino-terminal part of the PTH molecule.

It is important to note that both assays were able detect changes in PTH when blood ionized calcium was modified in the study of the PTH-Ca^{2+} curves (increase in PTH during hypocalcemia and decrease in PTH during hypercalcemia).

From a practical point it would be interesting to decide which assay should be used for the study of

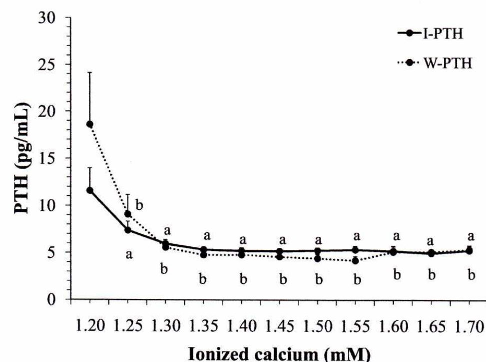

Fig. 4. The hypercalcemic part of the parathyroid hormone-extracellular calcium (PTH-Ca^{2+}) curve measured with the intact PTH (I-PTH) assay and the whole PTH (W-PTH) assay. Both curves were obtained after inducing hypercalcemia to 13 cats (see Materials and Methods for details). [a] and [b] = $P < 0.05$ vs PTH values at baseline calcium when measured with I-PTH (a) or W-PTH (b). Values are means ± standard error.

clinical cases and for research purposes in cats. Because the performance of the I-PTH and W-PTH assays is good and similar, both should be considered valid. The main advantage of the I-PTH assay is that it is more widely used in human and veterinary medicine and thus is more available for the measurement of individual samples in a clinical setting. However, because the activity of the PTH molecule is caused by the amino-terminal sequence of the molecule [22], W-PTH values should reflect more accurately PTH activity than I-PTH values. This contention is supported by the fact that W-PTH values in cats are more similar to reported PTH values in other species (human, dogs, rabbits, rats, etc.). In contrast, I-PTH values in cats are quite low (many of the I-PTH values measured in healthy cats would be considered abnormal in other species).

This is the first report that describes in detail the response of PTH to hypo- and hypercalcemia in cats. In the present work we decided to use both assays for the study of the PTH-Ca^{2+} curve because we were expecting to find additional insights in the dynamics of PTH secretion. The shape of the PTH-Ca^{2+} curve obtained with the two assays is practically identical, providing further support to the concept that both assays are valid for measurement of feline PTH. Basal and maximal W-PTH are greater than the corresponding values of I-PTH; however, minimal W-PTH and I-PTH are similar. The differences between W-PTH and I-PTH along the PTH-Ca^{2+} curve, which could be explained by secretion of different PTH fragments at different calcium concentrations, are best observed when studying the W-PTH/I-PTH ratio. This ratio increases in response to hypocalcemia (when more "active" W-PTH is needed) and decreases during hypercalcemia (when less active W-PTH is required). This finding has been previously reported by our laboratory in horses [4]. However, in dogs, a species in which I-PTH and W-PTH are very similar to that in humans, we were unable to find significant changes in the ratio during hypocalcemia [5].

Comparison of the feline PTH-Ca^{2+} curve with other species shows remarkable similarity to the canine PTH-Ca^{2+} curve [13,23,24], especially when the W-PTH values are considered. Basal, maximal, and minimal W-PTH values in cats are similar to the values reported in dogs. This is important because the dog has been extensively used as an animal model for the study of PTH and thus much of the information gathered in dogs could be potentially extrapolated to cats. Feline PTH-Ca^{2+} curve also bear great similarity to the curve reported in humans [12]. Although the shape of the curve is also similar in horses and rabbits, because baseline calcium is much greater in those species, parameters such as the SP are quite different [3,16].

In conclusion, the results of our study show that the I-PTH and W-PTH assays can be used for measurement of feline PTH. W-PTH concentration, which is likely to represent the true PTH concentration, is greater than I-PTH concentration in normal cats. Accuracy, precision, sensibility, reproducibility, and ability to detect changes in PTH in response to changes in calcium are similar in W-PTH and I-PTH assays. The PTH-Ca^{2+} curve in cats is similar to the canine curve and thus the extensive knowledge on dynamics of PTH secretion in dogs that can be found in the literature could be extrapolated to cats.

Acknowledgments

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References

[1] Barber PJ. Disorders of the parathyroid glands. J Feline Med Surg 2004;6:259–69.

[2] Schoenmakers I, Nap RC, Mol JA, Hazewinkel HAW. Calcium metabolism: an overview of its hormonal regulation and interrelation with skeletal integrity. Vet Q 1999;21:147–53.

[3] Estepa JC, Aguilera-Tejero E, Mayer-Valor R, Almaden Y, Felsenfeld AJ, Rodriguez M. Measurement of parathyroid hormone in horses. Equine Vet J 1998;30:476–81.

[4] Estepa JC, Garfia B, Gao P, Cantor T, Rodriguez M, Aguilera-Tejero E. Validation and clinical utility of a novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone in the horse. Equine Vet J 2003;35:291–5.

[5] Estepa JC, Lopez I, Felsenfeld AJ, et al. Dynamics of secretion and metabolism of PTH during hypo- and hypercalcaemia in the dog as determined by the "intact" and "whole" PTH assays. Nephrol Dial Transplant 2003;18:1101–7.

[6] Martin KJ, Hruska KA, Freitag JJ, Klahr S, Slatopolsky E. The peripheral metabolism of parathyroid hormone. N Engl J Med 1979;301:1092–8.

[7] Brown RC, Aston JP, Weeks I, Woodhead JS. Circulating intact parathyroid hormone measured by a two-site immunochemiluminometric assay. J Clin Endocrinol Metab 1987;65:407–14.

[8] Nussbaum SR, Zahradnik RJ, Lavigne JR, et al. Highly sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. Clin Chem 1987;33:1364–7.

[9] Barber PJ, Elliott J, Torrance AG. Measurement of feline intact parathyroid hormone: assay validation and sample handling studies. J Small Anim Pract 1993;34:614–20.

[10] Gao P, Scheibel S, D'Amour P, et al. Development of a novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone 1-84: implications for improvement of accurate assessment of parathyroid function. J Bone Miner Res 2001;16:605–14.

[11] Brown EM. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. J Clin Endocrinol Metab 1983;56:572–81.

[12] Felsenfeld AJ, Rodríguez M, Aguilera-Tejero E. Dynamics of parathyroid hormone secretion in health and secondary hyperparathyroidism. Clin J Am Soc Nephrol 2007;2:1283–305.

[13] Aguilera-Tejero E, Sánchez J, Almadén Y, Mayer-Valor R, Rodríguez M, Felsenfeld AJ. Hysteresis of the PTH-calcium curve during hypocalcemia in the dog: effect of the rate and linearity of calcium decrease and sequential episodes of hypocalcemia. J Bone Miner Res 1996;11:1226–33.

[14] Toribio RE, Kohn CW, Sams RA, Capen CC, Rosol TJ. Hysteresis and calcium set-point for the calcium parathyroid hormone relationship in healthy horses. Gen Comp Endocrinol 2003;130:279–88.

[15] Mayer GP, Hurst JG. Sigmoidal relationship between parathyroid hormone secretion rate and plasma calcium concentration in calves. Endocrinology 1978;102:1036–42.

[16] Bas S, Bas A, Estepa JC, Mayer-Valor R, Rodriguez M, Aguilera-Tejero E. Parathyroid gland function in the uremic rabbit. Domest Anim Endocrinol 2004;26:99–110.

[17] Felsenfeld AJ, Llach F. Parathyroid gland function in chronic renal failure. Kidney Int 1993;43:771–89.

[18] Toribio RE, Kohn CW, Chew DJ, Capen CC, Rosol TJ. Cloning and sequence analysis of the complementary DNA for feline preproparathyroid hormone. Am J Vet Res 2002;63:194–7.

[19] Bell O, Silver J, Naveh-Many T. Parathyroid hormone, from gene to protein, In: Naveh-Many T, ed. *Molecular Biology of the Parathyroid.* New York, NY: Kluwer Academic/Plenum Publishers; 2005:8–28.

[20] Torrance AG, Nachreiner R. Human-parathormone assay for use in dogs: validation, sample handling studies, and parathyroid function testing. Am J Vet Res 1989;50:1123–7.

[21] Potts JR, Bringhurst FR, Gardella T, Nussbaum S, Segre G, Kronenberg H. Parathyroid hormone: physiology, chemistry, biosynthesis, secretion, metabolism and mode of action, In: DeGroot LJ, editor. *Endocrinology*. 3rd ed. Philadelphia: W. B. Saunders; 1995:920–66.

[22] Tregear GW, Van Rietschoten J, Greene E, et al. Bovine parathyroid hormone: minimum chain length of synthetic peptide required for biological activity. Endocrinology 1973;93:1349–53.

[23] Sánchez J, Aguilera-Tejero E, Estepa JC, Almadén Y, Rodríguez M, Felsenfeld AJ. A reduced PTH response to hypocalcemia after a short period of hypercalcemia: a study in dogs. Kidney Int Suppl 1996;57:S18–22.

[24] Estepa JC, Aguilera-Tejero E, Almaden Y, Rodriguez M, Felsenfeld AJ. Effect of rate of calcium reduction and a hypocalcemic clamp on parathyroid hormone secretion: A study in dogs. Kidney Int 1999;55:1724–33.

SCIENTIFIC ARTICLE #2

Pineda C, Aguilera-Tejero E, Guerrero F, Raya AI, Rodriguez M, Lopez I. Mineral metabolism in growing cats: changes in the values of blood parameters with age. *J Feline Med Surg* 2013; 15: 866-871

Original Article

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Mineral metabolism in growing cats: changes in the values of blood parameters with age

Carmen Pineda[1], Escolastico Aguilera-Tejero[1], Fatima Guerrero[1], Ana I Raya[1], Mariano Rodriguez[2] and Ignacio Lopez[1]

Abstract

The purpose of this study was to describe changes in calcium, phosphorus, magnesium, parathyroid hormone, calcitriol and calcidiol in cats from 3 to 15 months of age. Fourteen European shorthair healthy cats of both sexes (seven males, seven females) belonging to a research colony were studied from 3 to 15 months of age. Plasma concentrations of total calcium, ionised calcium, albumin, phosphorus, magnesium, intact parathyroid hormone (I-PTH), whole parathyroid hormone (W-PTH), calcidiol and calcitriol were measured at 3, 6, 9, 12 and 15 months of age. From 3 months of age to adulthood cats showed a decrease in calcium (both total and ionised), phosphorus and magnesium. No major changes in PTH were evident, although the ratio of W-PTH:I-PTH decreased significantly with age. A reciprocal change in vitamin D metabolites (decrease in calcitriol and increase in calcidiol) was identified during the growing process. Our results, showing changes in most parameters of mineral metabolism during growth, reinforce the need to use adequate age-related reference values for diagnostic purposes.

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Mineral metabolism in growing animals is subjected to changes derived from the different rates of bone growth along time. These changes are reflected in the blood concentrations of minerals (calcium, phosphorus and magnesium) and hormones that participate in their homeostasis [parathyroid hormone (PTH) and vitamin D]. Thus, blood parameters of mineral metabolism tend to be different in the young growing animal and in the adult.[1]

Plasma calcium and phosphorus have been reported to be higher in young animals along growth phases because of increased bone turnover.[2–4] Calcium concentrations are greatest in puppies younger than 8 weeks of age and then decrease to adult levels at about 1 year of age.[3] Kittens have also been reported to have higher calcium concentrations before 8 weeks of age.[4] In puppies, phosphorus concentrations are increased above adult levels throughout the growth process and adult concentrations are reached at approximately 1 year of age.[3] The age effect is supposed to be less pronounced in cats, but immature cats also tend to have higher serum phosphorus concentrations.[4] Age variations have not been described for serum magnesium concentrations in puppies or kittens.[2]

In contrast with the minerals, changes in calciotropic hormones during growth have not been studied with detail in domestic carnivores. Two major hormones, PTH and 1,25-dihydroxyvitamin D (calcitriol) are involved in bone remodelling during growth. In humans, several studies have reported an increase of serum PTH concentrations throughout childhood and adolescence.[5,6] Calcitriol concentration is also elevated significantly in infants and adolescents when compared with adult standards.[7] However, serum concentrations of the precursor of calcitriol, 25-hydroxyvitamin D (calcidiol) are almost identical in infants, children, adolescents and adults.[8] The elevated calcitriol concentration during infancy and adolescence correlates with increased

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growth rates and, presumably, functions to enhance intestinal mineral absorption for rapid growth.[9]

In cats, knowledge about blood parameters related to mineral metabolism is scanty and there are only a few reports about changes in plasma minerals with growth.[2,4] Moreover, apart from scattered measurements obtained in healthy young animals to compare with clinical cases,[10] no information is available on changes in calciotropic hormones in growing cats.

We hypothesised that significant changes in the values of blood parameters related to mineral metabolism should be present along the growing process of the cat. Thus, the purpose of the work reported here was to describe changes in calcium, phosphorus, magnesium, PTH, calcitriol and calcidiol in cats from the age of 3 to 15 months, which was defined as adulthood.

Materials and methods

Fourteen European shorthair cats of both sexes (seven males, seven females) were studied. All cats were sexually intact. Cats were kept in a cattery belonging to the Animal House Facility of the University of Cordoba and were socialised by frequent human contact to prevent stress associated with handling. The animals had been monitored since birth and, at the time of entry in the experiment (age = 3 months), were subjected to a detailed physical examination and a blood analysis (complete blood count and biochemical profile) to assure clinical normality. All experimental procedures were approved by the ethics committee of the University of Cordoba.

Study design

After weaning, cats were raised on a commercial dry food for kittens (BabyCat; Royal Canin) containing 1.1% calcium, 0.97% phosphorus and 700 IU/kg vitamin D. At the time the experiments were started cats were switched to a junior diet (Kitten Advance; Affinity-Petcare SA) with the following content: 1.2% calcium, 1% phosphorus and 1500 IU/kg vitamin D. They were maintained on this diet for the whole experimental protocol. The first blood samples were obtained when the cats were 3 months old; subsequently, cats were sampled every 3 months until the age of 15 months. Blood samples were obtained with minimal restrain from the jugular vein using heparinised syringes and were handled anaerobically.

Analytical methods

Blood ionised calcium (iCa) was measured immediately after collection using selective electrodes (Bayer Diagnostics); then, samples were centrifuged and plasma was frozen at –20ºC. No changes in the analytes under study have been reported after freezing for 2 months at –20ºC.[11–13] Total calcium (tCa), phosphorus, magnesium and albumin were quantified by spectrophotometry (BioSystems SA). These techniques are used routinely in our hospital for the measurement of feline samples. PTH was measured in plasma samples within 2 months of collection using an immunoradiometric assay (IRMA) (Duo PTH Kit; Scantibodies Laboratory). This kit is designed for the quantitative determination of human 'whole' PTH (W-PTH) and 'intact' PTH (I-PTH), and has been recently validated by our laboratory for measurement of feline PTH.[14] The kit contains two IRMA assays. Both assays share a polyclonal antibody (anti-PTH 39-84) coated on to the surface of polystyrene beads as a solid phase. The IRMA for W-PTH uses a tracer antibody directed against the most N-terminal PTH (1-4) region. The use of this antibody guarantees that only biologically active PTH is detected. The IRMA for I-PTH uses a specific polyclonal antibody directed against PTH (7-34) as a tracer. With this antibody both W-PTH (1-84) and N-truncated PTH fragments (7-84) are detected. In addition to W-PTH and I-PTH measurements, the ratio between both parameters (W-PTH:I-PTH ratio) was calculated. Calcitriol and calcidiol were determined in plasma samples using a radioimmunoassay (Immunodiagnostic Systems). Both kits are complete assay procedures intended for the extraction and quantification of calcitriol and calcidiol, respectively. Intra- and interassay variation for these assays is: calcitriol, 9% and 12% respectively; and calcidiol, 6% and 8%, respectively. The sensitivity of the assays is <3 pg/ml for calcitriol and <2 ng/ml for calcidiol. Both assays showed linearity during dilution of feline plasma samples and the percentages of spiking recovery were 90 ± 6 % (calcitriol) and 107 ± 3 % (calcidiol). No matrix effect was detected when comparing dilutional parallelism of feline and human plasma samples.

Statistical analysis

Statistical analysis was performed by using the computer package SPSS for Windows 15.0. A Kolmogorov–Smirnov test was carried out to test for normality. All data sets, except albumin and PTH (I-PTH and W-PTH), passed normality testing. The effect of time on the normally distributed variables was assessed by repeated measures of analysis of variance using a general linear model with Fisher's least significant difference as a post hoc test. Kruskal–Wallis analysis followed by a post hoc rank total test was used for albumin and PTH. *P*-values <0.05 were considered significant. Values are given as the mean ± standard error.

Results

Total calcium concentration ranged between 9.8 and 10.2 mg/dl and did not change along the first 9 months of the study. At 12 months tCa started to decrease and was significantly lower at 15 months of age (8.6 ± 0.3 mg/dl). In contrast with tCa, a constant trend to decrease in iCa was

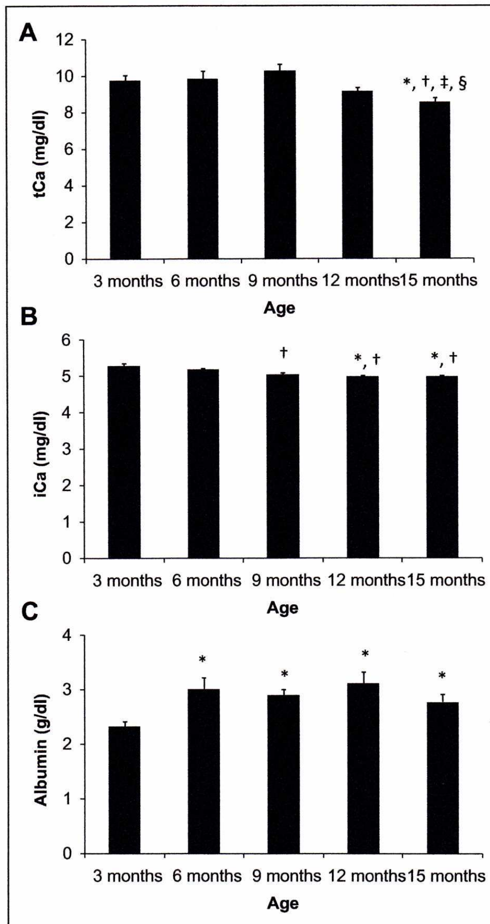

Figure 1 Plasma concentrations of total calcium (tCa) (a), ionised calcium (iCa) (b) and albumin (c) in cats from 3 to 15 months of age. *P <0.05 versus 3 months, †P <0.05 versus 6 months, ‡P <0.05 versus 9 months, §P <0.05 versus 12 months

observed along the experiments. Younger cats had higher iCa concentrations (5.28 ± 0.08 mg/dl, at 3 months of age) and iCa decreased progressively during the growing period, reaching a minimum of 4.96 ± 0.04 mg/dl at 12 months. Ionised calcium concentration remained steady from 12 to 15 months of age. Albumin concentration was around 3 g/dl at all sampling times except at 3 months — at this age a significantly lower albumin concentration (2.3 ± 0.1 g/dl) was measured (Figure 1).

Plasma phosphorus concentration was stable between 3 and 9 months of age at values ranging from 7.6 to 7.9 mg/dl. A significant decrease in phosphorus concentration was first detected at 12 months, 6.9 ± 0.3 mg/dl, and the decrease was more accentuated at 15 months, 5.3 ± 0.1 mg/dl. Plasma magnesium also decreased with age, but, in contrast to phosphorus, lower values were only identified at 15 months (2.7 ± 0.1 mg/dl), while between 3 and 12 months magnesium concentrations ranged between 3.3 ± 0.3 and 3.7 ± 0.2 mg/dl (Figure 2).

Figure 3 depicts plasma PTH concentrations. Both I-PTH and W-PTH remained constant from 3 to 9 months of age: I-PTH in the region of 5 pg/ml and W-PTH around 10 pg/ml. A non-significant peak was identified at 12 months in I-PTH (7.4 ± 1.2 pg/ml) and W-PTH (13.9 ± 2.9 pg/ml), and was followed by a small decrease at 15 months: 6.9 ± 0.9 pg/ml and 10.8 ± 2.4 pg/ml, respectively. A significant (P = 0.027) decline in the ratio W-PTH:I-PTH was observed with ageing (from 1.81 ± 0.14 at 3 months to 1.47 ± 0.15 at 15 months).

Calcitriol concentration was high at 3 and 6 months (180.3 ± 11 pg/ml and 182.9 ± 11.7 pg/ml, respectively). Plasma calcitriol concentration significantly decreased at 9 months (122.9 ± 15.7 pg/ml) and kept decreasing until 15 months (103.2 ± 11.5 pg/ml), although no significant differences were found between 9, 12 and 15 months of age. In contrast, plasma calcidiol concentration was significantly lower at 3 months of age (43.3 ± 7.4 ng/ml) when compared with other sampling times. Calcidiol concentrations recorded between 6 and 15 months were in the range of 68.2 ± 10.1 to 80.5 ± 10.6 ng/ml and did not differ when compared statistically (Figure 4).

Discussion

Concentrations of blood parameters related to mineral metabolism change along the growing process of the cat. From 3 months of age to adulthood, cats show a decrease in plasma minerals: calcium, phosphorus and magnesium; a decrease in calcitriol and an increase in calcidiol.

The adult (15 months) levels of all parameters under study were within the reference intervals of our laboratory for feline samples.

Kittens younger than 8 weeks have been reported previously to have higher plasma calcium than adults.[4] Our results show that between 3 and 12 months of age tCa concentration is significantly higher than values measured at 15 months. Thus, it would seem that, as in other species,[3] adult levels are not reached until the cat is at least 1 year old. Moreover, the biologically active calcium fraction, iCa, which, to our knowledge, has not been studied in growing kittens, shows a steady decrease during the first year of life and stabilises between 12 and 15 months of age. The discrepancy in the decline of iCa and tCa at younger ages may be explained by changes in plasma albumin. Lower albumin concentrations, as measured in younger cats,[4] will result in lesser tCa

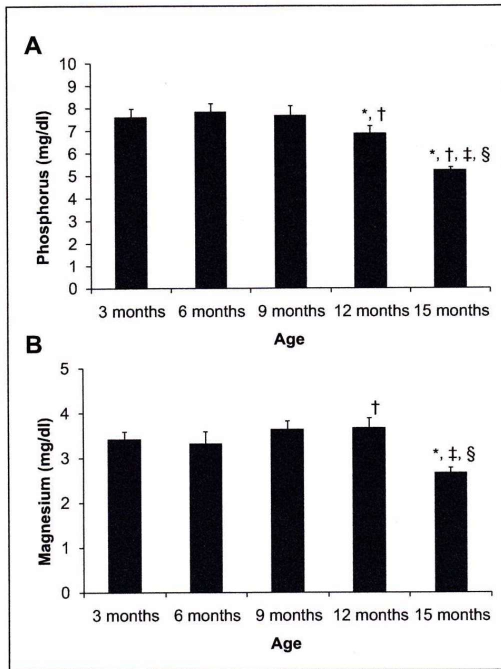

Figure 2 Plasma concentrations of phosphorus (a) and magnesium (b) in cats from 3 to 15 months of age. *P <0.05 versus 3 months, †P <0.05 versus 6 months, ‡P <0.05 versus 9 months, §P <0.05 versus 12 months

values and thus may explain why tCa is proportionally lower than iCa at these young ages.

In our study a decrease in both plasma phosphorus and magnesium concentrations, which follows the same time-frame than the decline in tCa (reduction at 12–15 months) has been identified. Previous studies have also reported that immature cats tend to have higher serum phosphorus concentrations.[4] However, age variations for serum magnesium concentrations in kittens had not been reported previously.[2]

Plasma PTH concentrations — both I-PTH and W-PTH — remained stable along the study period with only a small peak, which does not seem relevant for diagnostic purposes, around 1 year of age. The only reference to PTH values in growing cats that we have been able to find was provided by Tomsa et al[10] who measured I-PTH in seven healthy cats 4–6 months old and used them as control values to compare with cases of nutritional secondary hyperparathyroidism. The I-PTH values reported by Tomsa et al[10] are comparable with the I-PTH concentrations measured in our study. As described previously in adult cats,[14] W-PTH concentrations are also higher than I-PTH concentrations in growing kittens. We have reported an increase in the ratio W-PTH:I-PTH in hypocalcaemic adult cats, supporting the notion that this ratio tends to increase when more 'active' PTH is needed.[14] Thus, although these ratios should be interpreted with caution when using heterologous assays, the decline in the ratio W-PTH:I-PTH with age detected in kittens would be consistent with a decrease in the need for 'active' PTH once the skeletal growth is completed.

In our study reciprocal changes in calcidiol and calcitriol have been identified. These changes may be explained by the vitamin D requirements during growth. Our interpretation, based on the knowledge about calcitriol and calcidiol metabolism obtained in other species,[8,15,16] is that at younger ages the pro-vitamin D, calcidiol, is lower because it is being used to produce calcitriol, and calcitriol is higher to facilitate skeletal growth. When the growing needs decrease calcitriol concentration also declines and, owing to reduced demand, calcidiol increases.

In addition to the fluctuations in calcitriol and calcidiol concentrations detected during growth, it is very important to discuss their absolute values because they are clinically relevant in the diagnosis of juvenile bone disease. Evaluation of calcitriol and calcidiol concentrations is essential in the diagnosis of vitamin D-dependent rickets.[17–24] Two major subtypes of vitamin D-dependent rickets have been reported in kittens: type I and type II. Type I is due to a genetic defect affecting 1-alpha-hydroxylase, the enzyme that converts calcidiol to calcitriol,[17] while type II is associated with mutations in the gene encoding the vitamin D receptor (VDR).[18–21] In type I rickets calcitriol is decreased because it cannot be synthesised and the substrate (calcidiol) is increased, while in type II rickets calcitriol is increased because there is tissue resistance owing to the VDR defect, and calcidiol is either normal or decreased.[24]

As the diagnosis of feline vitamin D-dependent rickets is based in the evaluation of calcitriol and calcidiol plasma concentrations, it is obvious that to provide a correct interpretation of vitamin D status reference values adequate to the age of the kitten are needed. However, to our knowledge, these reference intervals have not been available before and most authors have likely used adult reference values. Our results seem to indicate that calcitriol values in growing kittens are higher than previously thought.

Reference intervals for calcidiol used previously in the diagnosis of rickets are more similar to our results. Our data are quite comparable to the results reported by Morris et al,[25] who investigated the effect of dietary vitamin D content on plasma calcidiol concentrations. They also found a tendency of increasing calcidiol with age, and this tendency was more accentuated when diets with abnormally high vitamin D content were fed.

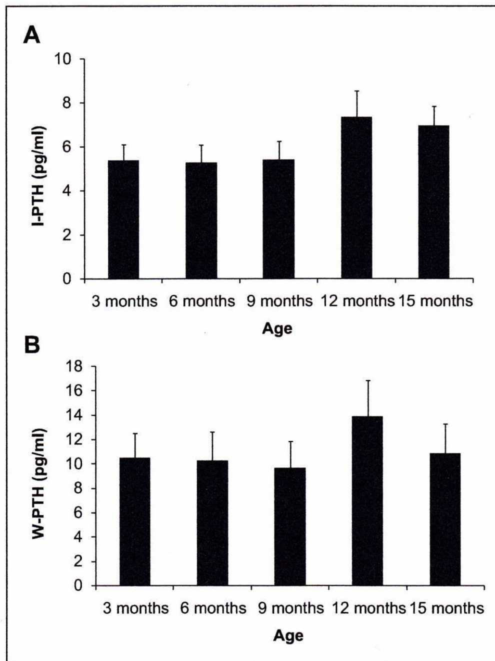

Figure 3 Plasma concentrations of intact parathyroid hormone (I-PTH) (a) and whole parathyroid hormone (W-PTH) (b) in cats from 3 to 15 months of age

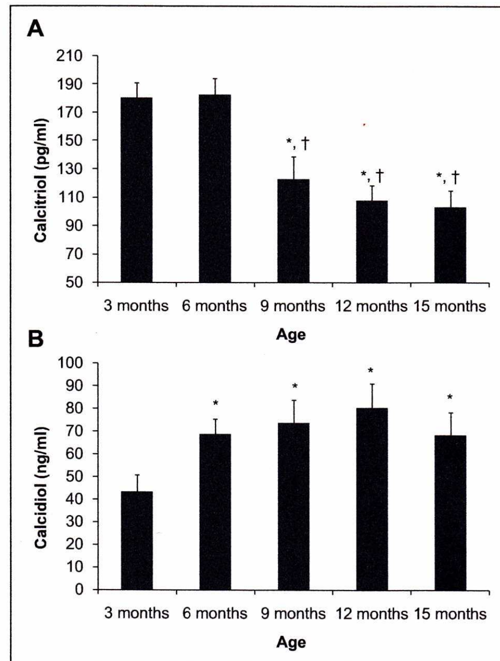

Figure 4 Plasma concentrations of calcitriol (a) and calcidiol (b) in cats from 3 to 15 months of age. $^{*}P <0.05$ versus 3 months, $^{\dagger}P <0.05$ versus 6 months

Thus, the main contribution of our work is to provide, for the first time, values for calcitriol and calcidiol in growing kittens at different stages of skeletal maturity. In addition, given the fact that the concentrations of these metabolites change with growth, when used for diagnostic purposes, values should be compared with the range corresponding to the age of the kitten. There are insufficient animals in this study to determine a reference interval. The animals were also a specific non-neutered population that lived in a controlled environment with standardised feeding regimes, so the values obtained should not be extrapolated to other populations. The principles established regarding the changes in parameters during the growth period apply to young cats, but reference intervals for this age group need to be developed by any laboratory performing these tests on their specific populations of young cats.

Conclusions

From 3 months of age to adulthood, cats show a decrease in plasma minerals: calcium (both tCa and iCa), phosphorus and magnesium. No major changes in PTH are evident, although the W-PTH:I-PTH ratio decreases significantly with age. A reciprocal change in vitamin D metabolites (decrease in calcitriol and increase in calcidiol) has been identified during the growing process. Our results reinforce the need to use adequate age-related reference values for diagnostic purposes, especially when evaluating the vitamin D status in the diagnosis of feline rickets.

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Conflict of interest The authors do not have any potential conflicts of interest to declare.

References

1 Schoenmakers I, Nap RC, Mol JA, et al. **Calcium metabolism: an overview of its hormonal regulation and interrelation with skeleteal integrity.** *Vet Q* 1999; 21: 147–153.
2 Kraft W, Hartmann K and Dereser R. **Age dependency of laboratory values in dogs and cats. Part II: Serum electrolytes.** *Tierarztl Prax* 1996; 24: 169–173 [in German].
3 Harper EJ, Hackett RM, Wilkinson J, et al. **Age-related variations in hematologic and plasma biochemical test results in Beagles and Labrador Retrievers.** *J Am Vet Med Assoc* 2003; 223: 1436–1442.

4 Levy JK, Crawford PC and Werner LL. **Effect of age on reference intervals of serum biochemical values in kittens.** *J Am Vet Med Assoc* 2006; 228: 1033–1037.
5 Arnaud SB, Goldsmith RS, Stickler GB, et al. **Serum parathyroid hormone and blood minerals: interrelationships in normal children.** *Pediatr Res* 1973; 7: 485–493.
6 Root AW and Harrison HE. **Recent advances in calcium metabolism. I. Mechanisms of calcium homeostasis.** *J Pediatr* 1976; 88: 1–18.
7 Chesney RW, Rosen JF, Hamstra AJ, et al. **Serum 1,25 dihydroxyvitamin D levels in normal children and in vitamin D disorders.** *Am J Dis Child* 1980; 134: 135–139.
8 Kano K, Yoshida H, Yata J, et al. **Age and seasonal variations in the serum levels of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D in normal humans.** *Endocrinol Jpn* 1980; 27: 215–221.
9 Lichtenstein P, Specker BL, Tsang RC, et al. **Calcium-regulating hormones and minerals from birth to 18 months of age: a cross-sectional study. I. Effects of sex, race, age, season, and diet on vitamin D status.** *Pediatrics* 1986; 77: 883–890.
10 Tomsa K, Glaus T, Hauser B, et al. **Nutritional secondary hyperparathyroidism in six cats.** *J Small Anim Pract* 1999; 40: 533–539.
11 Torrance AG and Nachreiner R. **Human-parathormone assay for use in dogs: validation, sample handling studies, and parathyroid function testing.** *Am J Vet Res* 1989; 50: 1123–1127.
12 Unterer S, Lutz H, Gerber B, et al. **Evaluation of an electrolyte analyzer for measurement of ionized calcium and magnesium concentrations in blood, plasma, and serum of dogs.** *Am J Vet Res* 2004; 65: 183–187.
13 Agborsangaya C, Toriola AT, Grankvist K, et al. **The effects of storage time and sampling season on the stability of serum 25-hydroxyvitamin D and androstenedione.** *Nutr Cancer* 2010; 62: 51–57.
14 Pineda C, Aguilera-Tejero E, Raya AI, et al. **Feline parathyroid hormone: validation of hormonal assays and dynamics of secretion.** *Domest Anim Endocrinol* 2012; 42: 256–264.
15 Mundy GR and Guise TA. **Hormonal control of calcium homeostasis.** *Clin Chem* 1999; 45: 1347–1352.
16 Seino Y, Shimotsuji T, Yamaoka K, et al. **Plasma 1,25-dihydroxyvitamin D concentrations in cords, newborns, infants, and children.** *Calcif Tissue Int* 1980; 30: 1–3.
17 Geisen V, Weber K and Hartmann K. **Vitamin D-dependent hereditary rickets type I in a cat.** *J Vet Intern Med* 2009; 23: 196–199.
18 Schreiner CA and Nagode LA. **Vitamin D-dependent rickets type 2 in a four-month-old cat.** *J Am Vet Med Assoc* 2003; 222: 337–339.
19 Godfrey DR, Anderson RM, Barber PJ, et al. **Vitamin D-dependent rickets type II in a cat.** *J Small Anim Pract* 2005; 46: 440–444.
20 Tanner E and Langley-Hobbs SJ. **Vitamin D-dependent rickets type 2 with characteristic radiographic changes in a 4-month-old kitten.** *J Feline Med Surg* 2005; 7: 307–311.
21 MacKenzie JM, Crawford J and Ghantous S. **Successful therapy of vitamin D-dependant rickets in a kitten.** *J Am Anim Hosp Assoc* 2011; 47: 290–293.
22 Phillips AM, Fawcett AC, Allan GS, et al. **Vitamin D-dependent non-type 1, non-type 2 rickets in a 3-month-old Cornish Rex kitten.** *J Feline Med Surg* 2011; 13: 526–531.
23 Henik RA, Forrest LJ and Friedman AL. **Rickets caused by excessive renal phosphate loss and apparent abnormal vitamin D metabolism in a cat.** *J Am Vet Med Assoc* 1999; 215: 1644–1649.
24 Dittmer KE and Thompson KG. **Vitamin D metabolism and rickets in domestic animals: a review.** *Vet Pathol* 2011; 48: 389–407.
25 Morris JG, Earle KE and Anderson PA. **Plasma 25-hydroxyvitamin D in growing kittens is related to dietary intake of cholecalciferol.** *J Nutr* 1999; 129: 909–912.

SCIENTIFIC ARTICLE #3

Pineda C, Aguilera-Tejero E, Raya AI, Guerrero F, Rodriguez M, Lopez I. Assessment of calcitonin response to experimentally induced hypercalcemia in cats. *Am J Vet Res* 2013; 74: 1514-1521

Assessment of calcitonin response to experimentally induced hypercalcemia in cats

Carmen Pineda, DVM; Escolastico Aguilera-Tejero, PhD; Ana I. Raya, PhD; Fatima Guerrero, PhD; Mariano Rodriguez, PhD; Ignacio Lopez, PhD

Objective—To characterize the dynamics of calcitonin secretion in response to experimentally induced hypercalcemia in cats.

Animals—13 healthy adult European Shorthair cats.

Procedures—For each cat, the calcitonin response to hypercalcemia (defined as an increase in ionized calcium concentration > 0.3mM) was investigated by infusing calcium chloride solution and measuring circulating calcitonin concentrations before infusion (baseline) and at various ionized calcium concentrations. Calcitonin expression in the thyroid glands of 10 of the cats was investigated by immunohistochemical analysis.

Results—Preinfusion baseline plasma calcitonin concentrations were very low in many cats, sometimes less than the limit of detection of the assay. Cats had a heterogeneous calcitonin response to hypercalcemia. Calcitonin concentrations only increased in response to hypercalcemia in 6 of 13 cats; in those cats, the increase in calcitonin concentration was quite variable. In cats that responded to hypercalcemia, calcitonin concentration increased from 1.3 ± 0.3 pg/mL at baseline ionized calcium concentration to a maximum of 21.2 ± 8.4 pg/mL at an ionized calcium concentration of 1.60mM. Cats that did not respond to hypercalcemia had a flat calcitonin-to-ionized calcium concentration curve that was not modified by changes in ionized calcium concentration. A significant strong correlation (r = 0.813) was found between the number of calcitonin-positive cells in the thyroid gland and plasma calcitonin concentrations during hypercalcemia.

Conclusions and Clinical Relevance—Healthy cats had very low baseline plasma calcitonin concentrations. A heterogeneous increase in plasma calcitonin concentration in response to hypercalcemia, which correlated with the expression of calcitonin-producing cells in the thyroid, was identified in cats. (*Am J Vet Res* 2013;74:1514–1521)

Calcitonin participates in the control of extracellular calcium concentrations. In plasma, calcium is found in 3 fractions: protein-bound calcium, complexed calcium, and ionized calcium. The stimulation of the calcium-sensing receptor located in thyroid gland C cells by ionized calcium promotes calcitonin secretion. Calcitonin inhibits osteoclastic bone resorption, has some positive influence on renal calcium excretion, and in the long term, may impair intestinal calcium absorption.[1] Although the hypocalcemic role of calcitonin is very consistent in the species in which it has been studied, the relative importance of this hormone in calcium metabolism seems to be species specific. Thus, although calcitonin is very important in the regulation of mineral metabolism in some species (eg, rats), calcitonin seems to have a minor role in others (eg, humans).[2]

Information about calcitonin in domestic animals is fragmentary. A specific assay for quantification of canine calcitonin has been described,[3] and measurement of plasma calcitonin concentrations in horses has been recently reported,[4] but no similar data are available for cats, to our knowledge.

The relationship between circulating calcitonin and ionized calcium concentrations can be studied through creation of a calcitonin-to-ionized calcium concentration curve, which describes the response of calcitonin concentration to changes in extracellular ionized calcium concentration. Calcitonin-to-ionized calcium concentration curves have been studied in clinically normal and uremic rats[5–7] and in humans with chronic renal failure.[8] For both species, a sigmoidal calcitonin-to-ionized calcium concentration curve has been reported. Among domestic animals, increases in circulating calcitonin concentration secondary to acute increases in ionized calcium concentration in dogs[3] and horses[4] have been reported. Nevertheless, no information is available regarding the dynamics of calcitonin secretion in response to changes in extracellular ionized calcium concentration in cats. The purpose of the

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study reported here was to characterize the dynamics of calcitonin secretion in response to experimentally induced hypercalcemia and the related changes in extracellular ionized calcium concentration in cats.

Materials and Methods

Animals—Thirteen European Shorthair cats of both sexes (6 males and 7 females), 16 to 18 months of age, that weighed 3.8 ± 0.3 kg were included in the study. Cats were randomly chosen from 5 litters born in a research colony. Cats were kept in a cattery belonging to the Animal House Facility of the University of Cordoba and were fed a diet containing calcium (1.1%), phosphorus (1%), and vitamin D (1,500 U/kg). Cats were considered healthy on the basis of physical examination findings and results of hematologic assessment and plasma biochemical profile. In addition, blood samples were obtained from all cats to evaluate mineral metabolism and thyroid gland function by measuring circulating concentrations of calcium (total and ionized), phosphorus, parathyroid hormone, 25-hydroxyvitamin D (calcidiol), 1,25-dihydroxyvitamin D (calcitriol), and free thyroxine. To study the response to hypercalcemia (defined as an increase [from the preinfusion baseline value] in whole blood ionized calcium concentration > 0.3mM), cats were anesthetized with a combination of ketamine hydrochloride[a] (15 mg/kg, IM) and midazolam[b] (0.4 mg/kg, IM). Once the experiments were finished, some cats were used for another study (an acute surgical procedure) that required euthanasia. Samples of thyroid gland tissue were obtained after death from 10 of the cats in which the dynamics of calcitonin secretion had been studied. All experimental procedures were approved by the Ethics Committee of the University of Cordoba.

Assessment of calcitonin response to ionized hypercalcemia—For each cat, the calcitonin-to-ionized calcium concentration curve was obtained following IV infusion of calcium chloride solution.[c] A jugular vein and the contralateral cephalic vein were cannulated with 18- and 20-gauge catheters,[c] respectively. The cephalic venous catheter was used for calcium chloride infusion, and the jugular venous catheter was used for blood sample collection. Hypercalcemia was achieved by IV infusion of calcium chloride solution, started at 0 minutes, at a rate of 0.27 mEq of calcium/kg/h. Infusion of calcium chloride was increased every 5 minutes up to a final rate of 0.55 mEq of calcium/kg/h after 50 minutes. Before initiation of the calcium chloride infusion, 3 blood samples (1 mL/sample) were obtained from each cat to provide baseline data (ie, mean values of whole blood ionized calcium and plasma calcitonin concentrations); thereafter, 10 blood samples (1 mL/sample) were obtained from each cat. Each one of the 10 samples was collected every 5 minutes until the end of the experiments (at 50 minutes). This protocol was extrapolated from a previous study[6] of calcitonin-to-ionized calcium concentration curves in other species and from our investigation[9] of parathyroid hormone-to-ionized calcium concentration curves in cats.

Individual calcitonin-to-ionized calcium concentration curves were constructed by adjusting the hormone and ionized calcium concentrations of each cat to a sigmoidal equation. The plasma calcitonin concentrations at standardized ionized calcium concentrations (from 1.20 to 1.60mM at increments of 0.05mM) were extrapolated from these individual curves. This range of circulating calcium concentrations was based on extrapolation from calcitonin-to-ionized calcium concentration curves in other species, and the infusion protocol was designed to at least achieve the upper range (1.60mM). From the data for all 13 cats, mean plasma calcitonin concentrations at standardized ionized calcium concentrations were calculated and used to obtain the calcitonin-to-ionized calcium concentration curve for the entire group.

Variables derived from the calcitonin-to-ionized calcium concentration curves were as follows: baseline plasma calcitonin concentration (the calcitonin concentration before initiation of hypercalcemia), maximum plasma calcitonin concentration (the highest calcitonin concentration observed in response to an increase in whole blood ionized calcium concentration > 0.3mM), the ratio of baseline to maximum plasma calcitonin concentration (multiplied by 100% to obtain a percentage), and the set point of ionized calcium concentration (the whole blood ionized calcium concentration that causes calcitonin release at a rate that is 50% of the maximum rate).

Laboratory measurements—Blood samples were obtained under anaerobic conditions and contained in tubes with heparin.[d] Ionized calcium concentration and pH were measured in whole blood immediately after collection with selective electrodes[e]; then, samples were centrifuged at 2,200 X g for 10 minutes and plasma was frozen at –20°C.

Calcitonin concentration was measured in the feline plasma samples with a 2-site immunoradiometric assay designed for the quantitative determination of calcitonin concentration in human serum,[f] which uses 2 goat polyclonal antibodies against the calcitonin molecule, with no cross-reactivity with parathyroid hormone, thyroid-stimulating hormone, or calcitonin gene–related peptide. The calcitonin assay was validated for use in cats by assessment of its precision, specificity, and sensitivity. Feline blood samples from 15 additional client-owned healthy cats (owner consent provided) and 2 of the university-owned study cats with experimentally induced hypercalcemia were used in the validation study. When used with the feline plasma samples, the calcitonin assay had a limit of detection of 0.9 pg/mL. The assay had an intra-assay coefficient of variation of 12.8% for feline samples with apparently normal plasma calcitonin concentration (ie, samples from normocalcemic cats) and 4.5% for feline samples with high plasma calcitonin concentration (ie, samples from hypercalcemic cats). Interassay variation ranged from 18.6% to 13.3%. Specificity was assessed by dilutional parallelism. A predictable dilution pattern was observed in samples with high calcitonin concentration. The assay demonstrated adequate dilutional parallelism, with percentages of recovery ranging from 124% to 129%.

Plasma concentrations of 25-hydroxyvitamin D (calcidiol) and 1,25-dihydroxyvitamin D (calcitriol)

were measured with radioimmunoassays[g] that have been validated for cats.[10] Plasma parathyroid hormone concentration was measured with an immunoradiometric assay[f] designed for the quantitative determination of human whole parathyroid hormone and intact parathyroid hormone concentrations validated by our laboratory for measurement of feline parathyroid hormone concentration.[9] Plasma free thyroxine concentration was measured by radioimmunoassay.[h]

Histologic and immunohistochemical evaluation—Following euthanasia by an IV overdose of barbiturate solution, thyroid glands from 10 of the 13 cats were collected and fixed in neutral-buffered 10% formalin. Formalin-fixed samples were embedded in paraffin, sectioned at 5 μm, and stained with H&E stain.

For immunohistochemical evaluation by the avidin-biotin-peroxidase complex method, tissue sections were dewaxed and rehydrated. Endogenous peroxidase activity was exhausted by incubation of the sections with 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature (approx 25°C). The antigen retrieval method used was microwave heating in 0.01M citrate buffer (pH, 6). Sections were incubated at 4°C overnight (approx 18 hours) in a humid chamber with the primary polyclonal rabbit anti-human calcitonin antibody[i] diluted 1:200. This antibody has been used successfully to detect calcitonin in other animal species.[11] After primary incubation, slides were washed in PBS solution and incubated with a biotinylated secondary antibody, diluted 1:200, for 30 minutes at room temperature. After washes in PBS solution, samples were incubated with the avidin-biotin-peroxidase complex[j] for 1 hour at room temperature. All tissue sections were finally rinsed in PBS solution, incubated for 1 minute with chromogen solution,[j] and counterstained with hematoxylin. Cells that reacted were counted by 2 independent investigators (CP and AIR) who were masked to details about the experimental group from which the sample was obtained. Positive cells in 40 nonoverlapping fields of 0.20 mm^2 chosen randomly in 4 diagonally positioned squares were counted. The mean number of positive cells per field was also calculated. For negative controls, nonimmune serum was used in place of primary antibody.

Statistical analysis—Statistical analysis was performed with statistical software.[k] Variables were normally distributed. For cats with plasma calcitonin concentrations below the limit of assay detection, a value of 0.9 pg/mL was assigned and used for statistical analysis. Plasma calcitonin concentrations at various ionized calcium concentrations were compared with hormonal concentrations at baseline ionized calcium concentration by means of paired *t* tests. Comparisons between subgroups of cats with different calcitonin responses to hypercalcemia (ie, responders vs nonresponders) were made by means of unpaired *t* tests. The Pearson test was used to determine correlation. Values of $P < 0.05$ were considered significant. Results are expressed as the mean ± SE.

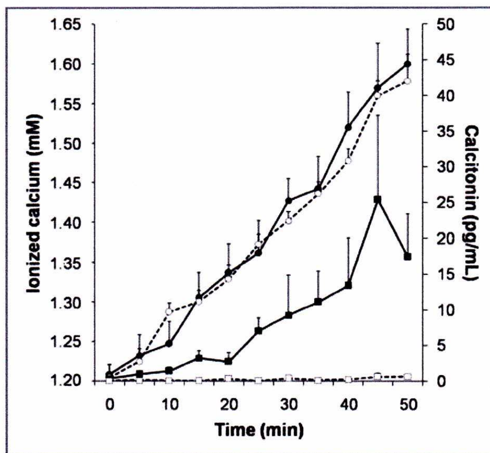

Figure 1—Mean ± SE whole blood ionized calcium concentration (circles) and plasma calcitonin concentration (squares) in 13 cats that received an IV infusion of calcium chloride (started at 0 minutes) at a rate of 0.27 mEq of calcium/kg/h, which was then increased every 5 minutes up to a final rate of 0.55 mEq of calcium/kg/h after 50 minutes, to induce hypercalcemia (defined as an increase in whole blood ionized calcium concentration > 0.3mM). Cats were assigned to 1 of 2 subgroups on the basis of whether their plasma calcitonin concentration did (responders [n = 6]; black symbols) or did not (nonresponders [7]; white symbols) increase in response to hypercalcemia.

Results

Baseline plasma calcitonin concentrations—Measurement of plasma calcitonin concentration in baseline samples ranged from less than the limit of detection (< 0.9 pg/mL) to 3.2 pg/mL. For the 11 cats with calcitonin concentrations below the limit of assay detection, a value of 0.9 pg/mL was assigned and used for statistical analysis. Among the 13 cats, the mean ± SE whole blood ionized calcium concentration was 1.20 ± 0.01 mM.

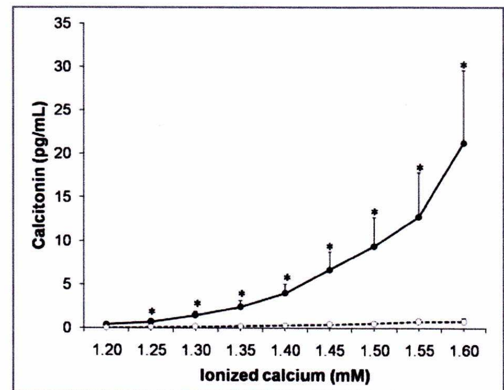

Figure 2—Calcitonin-to-ionized calcium concentration curve obtained after induction of hypercalcemia in the 13 cats in Figure 1. Plasma calcitonin concentration increased in response to the increasing severity of hypercalcemia in only 6 cats (responders [black circles]) and did not change in the remaining 7 cats (nonresponders [white circles]). Data are expressed as mean ± SE. *Value is significantly ($P < 0.05$) different from the plasma calcitonin concentration determined at the baseline whole blood ionized calcium concentration (ie, mean of the value determined from 3 samples obtained before infusion).

Plasma calcitonin concentrations during hypercalcemia—Plasma calcitonin concentrations were plotted against time (**Figure 1**) and against whole blood ionized calcium concentration (**Figure 2**). Examination of the calcitonin-to-ionized calcium concentration curve for individual cats revealed that the calcitonin response to hypercalcemia was varied. Plasma calcitonin concentration only increased in response to hypercalcemia in 6 of the 13 cats. In cats that responded to hypercalcemia (responders), basal calcitonin concentration increased from 1.3 ± 0.3 pg/mL (range, 0.9 to 3.2 pg/mL) at baseline ionized calcium concentration to a maximum calcitonin concentration of 21.2 ± 8.4 pg/mL (range, 8.0 to 43.5 pg/mL) at an ionized calcium concentration of 1.60mM. Nonresponders had a flat calcitonin-to-ionized calcium concentration curve that was not modified by changes in ionized calcium concentration. It is also interesting to note that a heterogeneous calcitonin response was observed in the subgroup of responders, with the maximum calcitonin concentration ranging from 8.0 to 43.5 pg/mL (**Figure 3**). Variables derived from the calcitonin-to-ionized calcium concentration curve were calculated on the basis of data from responders. The ratio of basal to maximum calcitonin concentration was 3.20 ± 1.45% (range, 0.02% to 9.09%), and the set point of the calcitonin-to-ionized calcium concentration curve was 1.46 ± 0.02mM (range, 1.40 to 1.52mM). A good correlation between ionized calcium and calcitonin concentrations ($r = 0.576$; $P < 0.001$) was found when the values obtained in normo- and hypercalcemic responders were pooled together.

To confirm that the lack of calcitonin response to hypercalcemia in some cats was not an erroneous finding, repeated calcitonin-to-ionized calcium concentration curves were determined for 6 cats (2 responders and 4 nonresponders). The calcitonin responses to hypercalcemia in the repeated experiments were consistent with the previous findings in each subgroup of cats

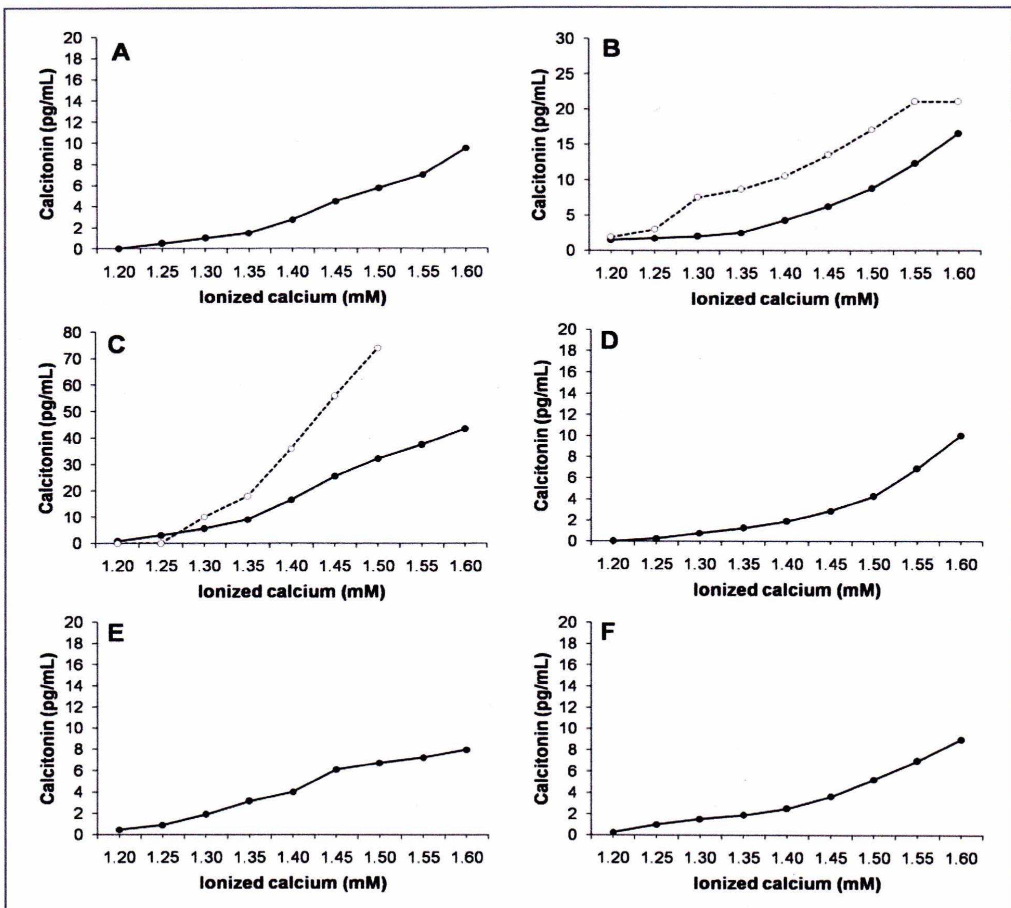

Figure 3—Individual calcitonin-to-ionized calcium concentration curves in the 6 cats that had an increase in plasma calcitonin concentration in response to hypercalcemia (responders [A through F]) in Figure 2. In 2 cats (B and C), the calcitonin-to-ionized calcium concentration curves were repeated to confirm the findings (white circles).

Table 1—Mean ± SE values of variables derived from the parathyroid hormone-to-ionized calcium concentration curve, other variables related to mineral metabolism, and free thyroxine concentration in 13 cats that received an IV infusion of calcium chloride at a rate of 0.27 mEq of calcium/kg/h, which was then increased every 5 minutes up to a final rate of 0.55 mEq of calcium/kg/h after 50 minutes, to induce hypercalcemia (defined as an increase in whole blood ionized calcium concentration > 0.3mM) and were assigned to 1 of 2 subgroups on the basis of whether their plasma calcitonin concentration did (responders) or did not (nonresponders) increase in response to hypercalcemia.

Variable	Responders (n = 6)	Nonresponders (n = 7)
Initial ionized calcium (mM)	1.20 ± 0.03	1.19 ± 0.01
Final ionized calcium (mM)	1.58 ± 0.04	1.58 ± 0.02
Difference (increase) in ionized calcium* (mM)	0.38 ± 0.03	0.39 ± 0.03
Initial intact parathyroid hormone (pg/mL)	10.4 ± 1.6	11.2 ± 2.5
Final intact parathyroid hormone (pg/mL)	5.9 ± 0.5	5.8 ± 0.5
Difference (decrease) in intact parathyroid hormone† (pg/mL)	4.5 ± 1.5	5.4 ± 2.5
Initial whole parathyroid hormone (pg/mL)	15.5 ± 3.7	16.5 ± 5.8
Final whole parathyroid hormone (pg/mL)	4.9 ± 0.5	4.4 ± 0.3
Difference (decrease) in whole parathyroid hormone† (pg/mL)	10.6 ± 3.4	12.2 ± 5.8
25-hydroxyvitamin D (ng/mL)	61.3 ± 8.0	58.0 ± 13.1
1,25-dihydroxyvitamin D (pg/mL)	117.1 ± 15.9	111.0 ± 12.4
Free thyroxine (ng/dL)	1.09 ± 0.10	1.12 ± 0.12

Concentrations of parathyroid hormone (intact and whole), 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and free thyroxine were measured in plasma samples; ionized calcium concentrations were measured in whole blood samples.

*The differences in ionized calcium concentration were calculated as final concentration minus initial concentration for each subgroup. †The differences in parathyroid hormone concentration were calculated as initial concentration minus final concentration for each subgroup.

(ie, for responders, calcitonin concentration increased during hypercalcemia, whereas for nonresponders, very low plasma calcitonin concentrations were maintained during hypercalcemia). Interestingly, in the second experiment, both responders had a greater calcitonin response than they did in the first experiment (Figure 3). The reason for this was unclear. It may have been simply coincidental, or it may have been reflecting an age-related effect. However, any effect related to the time elapsed between the 2 experiments was not evident in the nonresponders.

Additional biochemical evaluations—A set of plasma biochemical variables was measured before starting the experiments to ensure that all cats were healthy. In searching for an explanation for the heterogeneous calcitonin response to hypercalcemia, we investigated whether any difference in plasma biochemical variables could be related to the calcitonin response to hypercalcemia. The results indicated that no apparent difference in any of the multiple variables between responders and nonresponders was evident (**Table 1**). Moreover, no correlation was found between plasma concentrations of calcitonin and any of the plasma biochemical variables.

Histologic and immunohistochemical evaluations—Histologic evaluation of thyroid gland tissue from 6 responders and 4 nonresponders in which the dynamics of calcitonin secretion had been studied was performed. These cats were chosen because they were being used in a terminal surgical investigation, which did not involve the thyroid glands. No histopathologic lesions were found in any of the thyroid gland sections stained with H&E stain. Calcitonin was expressed in the cytoplasm of C cells, which was filled with positive immunoreactive substance. These cells were grouped in cell clusters (4 to 5 cells) or as single cells (**Figure 4**), which is similar to the results reported by Titlbach et al.[12] Interestingly, calcitonin-positive cells were identified in thyroid gland tissue from responders and nonresponders. However, both the total number of calcitonin-positive C cells and mean number of calcitonin-positive C cells per field were significantly ($P < 0.05$) higher in responders (342.8 ± 62.9 cells and 8.6 ± 1.6 cells/field, respectively) than in nonresponders (126.5 ± 31.1 cells and 3.2 ± 0.8 cells/field, respectively). In addition, a significant correlation ($r = 0.813$; $P = 0.004$) was found between maximum calcitonin concentration and both the total number of calcitonin-positive C cells and mean number of calcitonin-positive C cells per field.

Discussion

Abnormalities of ionized calcium metabolism (eg, hypercalcemia of malignancy and hypo- or hypercalcemia associated with renal failure and nutritional disorders) are common in cats.[13,14] Moreover, cats can develop derangements of ionized calcium metabolism, such as idiopathic hypercalcemia, with unknown etiopathogenesis.[15] To have a comprehensive understanding of these disorders, it is important to know the dynamics of secretion of calciotropic hormones. To our knowledge, this report is the first to describe in detail the response of calcitonin to experimentally induced hypercalcemia in cats.

In cats as well as in most domestic animals, endocrine studies are often complicated by the lack of assays with specific antibodies. Even though this dearth may be regarded as an important problem for quantification of calciotropic hormones in animals, it should be noted that calcitonin molecules are quite similar among different species.[16] Sequencing of calcitonin has revealed homology ranging from 53% to 90% among the mammals (humans, rats, dogs, and horses) in which it has been studied.[3,16,17] Thus, heterologous assays (ie, assays incorporating antibodies against the human calcitonin molecule) can be used to reliably measure calcitonin concentration in some other mammals. Recently, the usefulness of a human calcitonin assay for quantification of equine calcitonin concentration has been demonstrated.[4]

Very little information is available regarding plasma calcitonin concentrations in domestic animals, and to our knowledge, calcitonin concentrations in cats have not been reported. Our results show very low

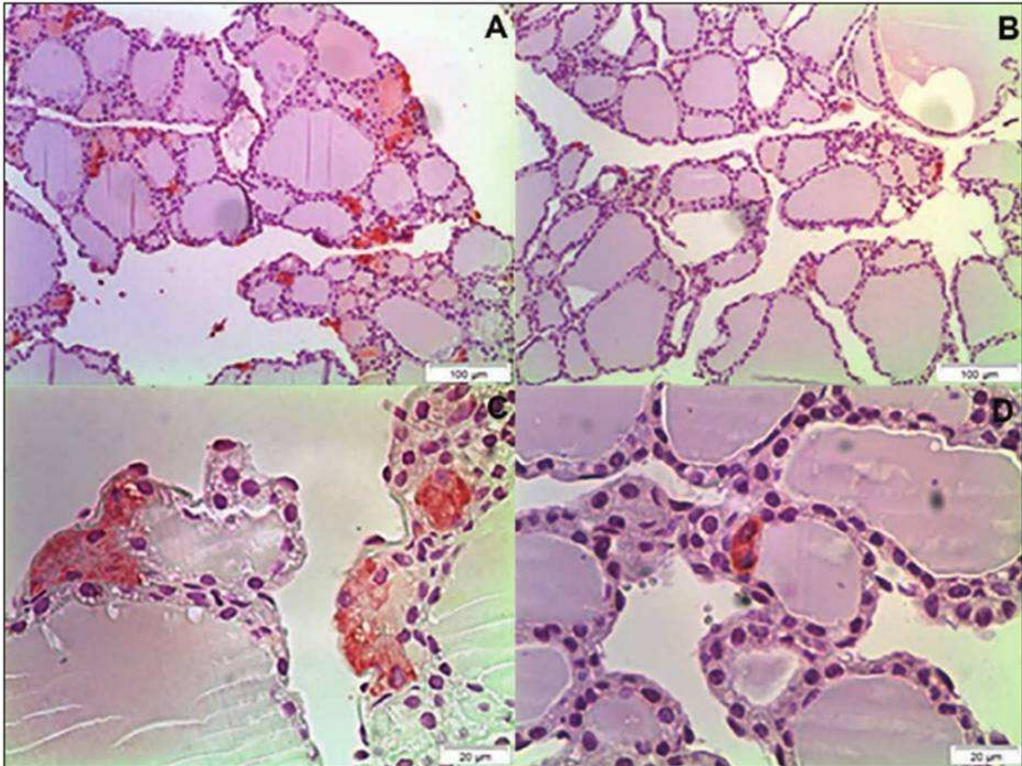

Figure 4—Representative photomicrographs of C cells immunolabeled with anti–human calcitonin antibody in sections of thyroid gland tissue samples obtained from 1 cat in which plasma calcitonin concentration did (responder [A and C]) or did not (nonresponder [B and D]) increase during hypercalcemia. In general, the number of calcitonin-positive cells in tissue sections obtained from responders (6 cats) was higher than that in tissue sections from nonresponders (4 cats). Immunohistochemical stain specific for calcitonin; bar in panels A and B = 100 µm, and bar in panels C and D = 20 µm.

calcitonin concentrations in clinically normal cats. Of note, it is not unusual to find low calcitonin concentrations in other species. In fact, healthy humans may have basal calcitonin concentrations similar to what we have found in cats (0.9 to 3.2 pg/mL) and nearly undetectable calcitonin concentrations are considered normal in humans.[18]

In domestic animals, the calcitonin response to changes in ionized calcium concentration has been studied in dogs and horses that received an IV bolus of calcium.[3,4] Basal circulating calcitonin and maximum calcitonin concentrations seem to be higher both in dogs[3] and horses[4] than in cats. Although previous studies[3,4] provide evidence of calcitonin response to changes in circulating calcium concentration in domestic animals, those data do not allow a thorough evaluation of the calcitonin-to-ionized calcium concentration curve. Thus, we had to compare results of the present study with data from rats, the only animal species for which a detailed calcitonin-to-ionized calcium concentration curve has been described.[5–7] The shape and the setpoint of the calcitonin-to-ionized calcium concentration curves for cats and rats are similar. However, basal calcitonin and maximum calcitonin concentration are much higher in rats than in cats. In addition, the heterogeneity in the calcitonin response to hypercalcemia has not been reported in other mammals, to our knowledge. The importance of calcitonin in the control of mineral metabolism is quite different between species. In rats, calcitonin seems to be very important for avoiding development of hypercalcemia. Results of 1 study[7] have indicated a tendency for rats to become hypercalcemic after thyroparathyroidectomy or after selective thyroidectomy. By contrast, in thyroidectomized cats, hypocalcemia is reportedly the main complication; hypocalcemia that develops in some cats after total thyroidectomy is assumed to be related to damage to the parathyroid glands.[19] Nonetheless, the fact that the ablation of thyroid gland tissue does not result in hypercalcemia suggests that calcitonin has less influence than parathyroid hormone on ionized calcium metabolism in cats. Taken together, the results of the present study (low baseline calcitonin concentration plus moderate and heterogeneous response to hypercalcemia) would support the contention that, in contrast to the case in rats, calcitonin seems to have a secondary role in calcium homeostasis in cats.

One of the more interesting findings of the present study was the fact that some cats did not have an increase in plasma calcitonin concentration in response to hypercalcemia. This surprising discovery was confirmed through repeated experiments. Given that plasma calcitonin concentrations were not increased at any time in nonresponders, the differences between the responders and nonresponders should be at the level of secretion rather than at the level of clearance. No significant differences were found in baseline calcitonin concentration between responders and nonresponders; however, considering that baseline calcitonin was very low in both subgroups, it would be unlikely to find such differences. When analyzing the ionized calcium concentration changes as a result of the calcium chloride infusion, it was interesting to note that there was no difference between the 2 subgroups of cats. The rate of calcium change, which has been shown to influence calcitonin secretion,[6] was almost identical in both responders and nonresponders (Figure 1). The mean ± SE difference in ionized calcium concentration (final concentration minus initial concentration) was 0.38 ± 0.03mM in responders and 0.39 ± 0.03mM in nonresponders. Thus, the absence of calcitonin secretion did not result in a more profound hypercalcemia in nonresponders. To determine whether the lack of calcitonin would be compensated by changes in parathyroid hormone concentration, final parathyroid hormone concentrations at the end of hypercalcemia and the difference in parathyroid hormone concentration (initial concentration minus final concentration) in both subgroups were compared. Again, no significant difference in either variable was found between responders and nonresponders on the basis of data obtained with intact or whole parathyroid hormone assays. Also, no differences in vitamin D status or in thyroid gland function were found between the 2 subgroups of cats. Immunohistochemical analysis of thyroid gland tissues revealed that the density of cells expressing calcitonin was higher in responders than in nonresponders. Thus, a histologic basis to explain the disparate response to hypercalcemia between the 2 subgroups of cats was found.

In humans, gender is known to affect the calcium response of calcitonin. Men have higher basal calcitonin concentrations and a more pronounced calcitonin response to hypercalcemia than women.[20] It is interesting to note that men have also been reported to have twice as many C cells as women.[21] In the present study in cats, the lack of calcitonin response to hypercalcemia was not related to sex, given that both males (n = 3) and females (4) were included among the nonresponders. However, we believe that the disparity in the number of calcitonin-positive C cells found between the subgroups of cats and its correlation with calcitonin response to hypercalcemia is the basis of the heterogeneity in calcitonin secretion found in the present study.

Although the data obtained in the present study seem to support a minor role of calcitonin in the control of ionized calcium concentration in cats, it must be noted that we evaluated healthy cats that were induced to develop acute hypercalcemia and that the situation may be different in cats with chronic hypercalcemia. Idiopathic hypercalcemia is rare in cats, and its pathophysiology remains unexplained.[15] Based on the findings of the present study, it could be rewarding to investigate plasma calcitonin concentrations in cats with idiopathic hypercalcemia. In a chronic situation, nonresponders may have limited capacity to counteract hypercalcemia, not only because of the lack of direct hypocalcemic effect of calcitonin but also because calcitonin is known to decrease the calcemic response to parathyroid hormone; thus, in the absence of calcitonin, parathyroid hormone would be expected to promote hypercalcemia.[22]

The results of the present study indicated that the calcitonin response to hypercalcemia in cats was heterogeneous. Two subgroups of cats were distinguished: cats in which plasma calcitonin concentration increased during hypercalcemia and cats in which calcitonin concentrations remained very low even at very high ionized calcium concentrations (up to 1.60mM) that are consistent with concentrations in cats with clinical hypercalcemia. Although no deleterious effects in the acute response to hypercalcemia were detected in the nonresponders, cats in which plasma calcitonin concentration did not increase could be predisposed to disorders of ionized calcium metabolism. Therefore, further investigation of this topic is warranted. In any case, additional studies exploring calcitonin concentrations in naturally hypercalcemic cats (idiopathic or otherwise) seem necessary.

a. Pfizer, Madrid, Spain.
b. Normon SA, Madrid, Spain.
c. B. Braun, Melsungen, Germany.
d. Greiner Bio-One, Kremsmünster, Austria.
e. Bayer Diagnostics, Barcelona, Spain.
f. Scantibodies Laboratory Inc, Santee, Calif.
g. Immunodiagnostic Systems Ltd, Boldon, Tyne and Wear, England.
h. Izotop, Budapest, Hungary.
i. DakoCytomation, Glostrup, Denmark.
j. Vector Laboratories Inc, Burlingame, Calif.
k. SPSS, version 15.0 for Windows, SPSS Inc, Chicago, Ill.

References

1. Schoenmakers I, Nap RC, Mol JA, et al. Calcium metabolism: an overview of its hormonal regulation and interrelation with skeletal integrity. *Vet Q* 1999;21:147–153.
2. Inzerillo AM, Zaidi M, Huang CL. Calcitonin: the other thyroid hormone. *Thyroid* 2002;12:791–798.
3. Hazewinkel HAW, Schoenmakers I, Pelling D, et al. Biological potency and radioimmunoassay of canine calcitonin. *Domest Anim Endocrinol* 1999;17:333–344.
4. Rourke KM, Kohn CW, Levine AL, et al. Rapid calcitonin response to experimental hypercalcemia in healthy horses. *Domest Anim Endocrinol* 2009;36:197–201.
5. Torres A, Rodriguez M, Felsenfeld A, et al. Sigmoidal relationship between calcitonin and calcium: studies in normal, parathyroidectomized, and azotemic rats. *Kidney Int* 1991;40:700–704.
6. Wang W, Lewin E, Olgaard K. Rate-dependency of calcitonin secretion in response to increased plasma Ca^{2+}. *Eur J Clin Invest* 2002;32:669–673.
7. Wang W, Lewin E, Olgaard K. Role of calcitonin in the rapid minute-to-minute regulation of plasma Ca^{2+} homeostasis in the rat. *Eur J Clin Invest* 2002;32:674–681.
8. Felsenfeld AJ, Machado L, Rodriguez M. The relationship between serum calcitonin and calcium in the hemodialysis patient. *Am J Kidney Dis* 1993;21:292–299.
9. Pineda C, Aguilera-Tejero E, Raya AI, et al. Feline parathyroid

hormone: validation of hormonal assays and dynamics of secretion. *Domest Anim Endocrinol* 2012;42:256–264.
10. Pineda C, Aguilera-Tejero E, Guerrero F, et al. Mineral metabolism in growing cats: changes in the values of blood parameters with age. *J Feline Med Surg* 2013;15:866–871.
11. Martín-Lacave I, Rojas F, Bernabe R, et al. Comparative immunohistochemical study of normal, hyperplastic and neoplastic C cells of the rat thyroid gland. *Cell Tissue Res* 2002;309:361–368.
12. Titlbach M, Velický J, Lhotová H. Prenatal development of the cat thyroid: immunohistochemical demonstration of calcitonin in the "C" cells. *Anat Embryol (Berl)* 1987;177:51–54.
13. Savary KCM, Price GS, Vaden SL. Hypercalcemia in cats: a retrospective study of 71 cases (1991–1997). *J Vet Intern Med* 2000;14:184–189.
14. Barber PJ, Elliott J. Feline chronic renal failure: calcium homeostasis in 80 cases diagnosed between 1992 and 1995. *J Small Anim Pract* 1998;39:108–116.
15. Midkiff AM, Chew DJ, Randolph JF, et al. Idiopathic hypercalcemia in cats. *J Vet Intern Med* 2000;14:619–626.
16. Mol JA, Kwant MM, Arnold ICJ, et al. Elucidation of the sequence of canine (pro)-calcitonin. A molecular biological and protein chemical approach. *Regul Pept* 1991;35:189–195.
17. Toribio RE, Kohn CW, Leone GW, et al. Molecular cloning and expression of equine calcitonin, calcitonin gene-related peptide-I, and calcitonin gene-related peptide-II. *Mol Cell Endocrinol* 2003;199:119–128.
18. Ramachandran R, Benfield P, Dhillo WS, et al. Need for revision of diagnostic limits for medullary thyroid carcinoma with a new immunochemiluminometric calcitonin assay. *Clin Chem* 2009;55:2225–2226.
19. Naan EC, Kirpensteijn J, Kooistra HS, et al. Results of thyroidectomy in 101 cats with hyperthyroidism. *Vet Surg* 2006;35:287–293.
20. Heath H III, Sizemore GW. Plasma calcitonin in normal man. Differences between men and women. *J Clin Invest* 1977;60:1135–1140.
21. Guyétant S, Rousselet MC, Durigon M, et al. Sex-related C cell hyperplasia in the normal human thyroid: a quantitative autopsy study. *J Clin Endocrinol Metab* 1997;82:42–47.
22. Rodriguez M, Felsenfeld AJ, Torres A, et al. Calcitonin, an important factor in the calcemic response to parathyroid hormone in the rat. *Kidney Int* 1991;40:219–225.

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Effects of calculolytic diets on feline mineral metabolism

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ABSTRACT
The influence of two calculolytic diets on mineral metabolism was evaluated in fourteen cats. Feeding the calculolytic diets for two months did not alter plasma concentrations of calcium (ionized and total), phosphorus, magnesium and parathyroid hormone. However, a marked decline in calcitriol was observed after administering both treatment diets: from 111.0 ± 11.7 to 91.1 ± 11.6 pg/mL (Diet 1) and from 110.4 ± 12.5 to 82.5 ± 9.6 pg/mL, $P<0.05$ (Diet 2). Cats fed Diet 1 showed a significant increase in urine Ca concentration (from 1.3 ± 0.1 to 1.9 ± 0.1 mg/dL). Magnesium concentration in urine was significantly increased with both diets, from 2.8 ± 0.3 to 4.0 ± 0.2 mg/dL (Diet 1) and from 2.7 ± 0.3 to 4.4 ± 0.6 mg/dL (Diet 2). Both diets resulted in an increased urinary concentration of magnesium, through different mechanisms: urine acidification (Diet 1) and increased sodium load (Diet 2).

Keywords: Calculolytic diet, Cats, Mineral metabolism

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1. Introduction

Nutritional management, through the use of calculolytic diets, is quite effective both in preventing and treating struvite uroliths in cats (Kerr, 2013). Diets for the prevention or treatment of struvite uroliths in cats are designed to modify urine by reducing pH (adding methionine, ammonium chloride, etc), and urinary minerals (reducing Mg, P) (Kerr, 2013). In addition, these diets usually include a high sodium chloride (NaCl) concentration, which is intended to increase water consumption (Kirk et al., 2006; Xu et al., 2009) and thus would increase the urine volume and can result in a higher dilution of the urine. Since calculolytic diets are fed for prolonged periods (months to years) they are potentially able to modify mineral metabolism and, in fact, a debate about their safety has been raised (Ching et al., 1989).

Acidosis is known to influence mineral metabolism in cats by increasing parathyroid hormone (PTH) secretion, promoting bone resorption, decreasing calcitriol (1,25 $(OH)_2$ D_3) concentrations in the blood and altering urinary handling of minerals (Ching et al., 1989). Lowering urine pH may increase the urinary excretion of minerals, like calcium (Ca) and magnesium (Mg) (Ching et al., 1989). However, although its etiopathogenesis is unknown, it has also been suggested that acidifying diets may be related to feline idiopathic hypercalcemia (Midkiff et al., 2000).

The effect of high dietary salt concentrations on feline mineral metabolism is controversial: while dietary NaCl has been reported to increase urinary fractional excretion (FE) of Ca (Kirk et al., 2006) other authors have not found effect of dietary salt on urinary Ca or Mg concentrations (Xu et al., 2009). Recent work in rats supports a link between salt consumption and renal handling of Ca and Mg, which is reflected by increases in PTH (Lee et al., 2012).

The objective of this study was to evaluate the influence of two calculolytic diets on parameters of mineral metabolism in cats that had received the diets over a period that is recommended by the manufacturers (two months). Our hypothesis was that these diets, which were formulated with low concentrations of specific minerals (Mg, P) and at the same time were designed to acidify urine and/or increase diuresis, would modify blood parameters of mineral metabolism.

2. Materials and methods

2.1 Animals

Fourteen sexually-intact European short-hair cats (7 males, 7 females), aged 19.2 ± 0.2 months and weighing 3.5 ± 0.2 Kg were studied. Cats were randomly chosen from five different litters born in a research colony and were kept in a cattery belonging to the Animal House Facility of the University of Cordoba. Cats were considered healthy on the basis of a normal physical examination, normal hemogram and normal biochemical profile. All experimental procedures were approved by the Ethics Committee of the University of Cordoba (File Number: 1320, Approval Date: February 2009).

2.2 Study Design

Two dry commercial diets designed for struvite urolith dissolution were evaluated: Diet 1 (Prescription Diet Feline s/d, Hill's, Madrid, Spain) and Diet 2 (Veterinary Diet Urinary S/O Feline, Royal Canin, Aimargues, France). Diet composition is shown in Table 1. The study was

Table 1
Composition based on dry matter (as supplied by the manufacturer) of the diets fed to the cats during the study.

Nutrients	Control diet	Diet 1	Diet 2
Protein (%)	35.0	34.5	34.5
Carbohydrate (%)	30.0	31.9	29.5
Lipid (%)	16.0	26.7	15.0
Fiber (%)	1.5	0.9	2.7
Metabolizable Energy (Kcal/Kg)	4180	4590	4070
Ca (%)	1.10	0.87	0.80
P (%)	1.00	0.71	0.70
Ca:P ratio (%)	1.10	1.23	1.14
Mg (%)	0.085	0.06	0.05
Na (%)	0.30	0.40	0.90
K (%)	0.80	0.95	1.00
Cl (%)	1.05	1.31	1.90
Vitamin D (μg/Kg)	37.5	14.7	19.1

Control diet, Advance Cat Adult Chicken & Rice, Affinity-Petcare, Barcelona, Spain; Diet 1, Prescription Diet Feline s/d, Hill's, Madrid, Spain; Diet 2, Veterinary Diet Urinary S/O Feline, Royal Canin, Aimargues, France; Ca, calcium; P, phosphorus; Mg, magnesium; Na, sodium; K, potassium; Cl, chloride.

designed as a two sequence, four period cross-over protocol with a baseline period in which a standard diet were fed, two 60 days 'run-in' periods, in which calculolytic diets (Diet 1 and Diet 2) were fed, and one 30 days 'wash-out' period in which the standard diet was fed.

Prior to commencement of the study, cats were fed a dry adult feline maintenance diet (Advance Cat Adult Chicken & Rice, Affinity-Petcare, Barcelona, Spain) for 6 months. The cats were routinely fed ad libitum.

At the beginning of the study cats were randomly divided into two groups each receiving one of the two treatment diets (Diet 1 and Diet 2) and they were fed for 60 days. Blood and urine were collected before starting feeding the treatment diets and after 30 and 60 days. The maintenance diet was then fed for one month during the 'wash-out' period. After the 'wash-out' period the two diets were reversed for the treatment groups during 60 additional days, following the same sampling schedule.

A full complete blood count, biochemical profile and urinalysis were performed at the beginning and at the end of the study, whereas a complete urinalysis with FE of Ca, Mg, P, Na, Cl and potassium (K), selected biochemical parameters (iCa, tCa, Mg, P, PTH, and calcitriol) and electrolytes (Na, K, Cl) were measured on day 30 and day 60 of the study. Results for the significant variables for each sequence were averaged for the two periods.

2.3 Laboratory measurements

Blood samples were obtained under anaerobic conditions in heparinized tubes (Greiner Bio-One, Kremsmunster, Austria). Plasma iCa, Na, K and Cl were measured in whole blood immediately after collection using

selective electrodes (Bayer Diagnostics, Barcelona, Spain); then, samples were centrifuged at 3500 rpm for 10 minutes and plasma was frozen at -20 ºC. Total Ca, P, Mg, creatinine and albumin were quantified by spectrophotometry (BioSystems SA, Barcelona, Spain).

Plasma concentrations of calcitriol were measured using a radioimmunoassay (Immunodiagnostic Systems Ltd, Boldon, UK) that has been validated for cats (Pineda et al., 2013). Parathyroid hormone was measured using an immunoradiometric assay (Scantibodies Laboratory Inc, Santee, CA, USA) designed for the quantitative determination of human "intact" PTH validated by our laboratory for measurement of feline PTH (Pineda et al., 2012).

Urine samples (approximately 5 mL) were obtained by cystocentesis. Immediately after collection, urinary pH (Panreac, Quimica S.A., Barcelona, Spain) and specific gravity (Zuzi, Auxilab S.L., Beriain, Spain) were measured. The urine was stored at -20 ºC before further analysis. Total Ca, P, Mg and creatinine were quantified by spectrophotometry (BioAssay Systems, Hayward, CA, USA (tCa) and BioSystems SA, Barcelona, Spain (P, Mg and creatinine). Na, K and Cl were quantified in urine samples by ion-selective indirect potentiometry (Abbott Diagnostics, Madrid, Spain). Fractional excretion for each electrolyte was calculated according to the following equation:

$$FE\ (\%) = [(U_e \times P_{creat}) / (P_e \times U_{creat})] \times 100,$$

where U_e is the urine electrolyte concentrations, P_{creat} is the plasma creatinine concentration, P_e plasma electrolyte concentration and U_{creat} is the urine creatinine concentration.

2.4 Statistical analysis

Statistical analysis was performed with statistical software (SPSS for Windows 15.0, Chicago, USA). A Kolmogorov-Smirnov test was carried out to test for normality. All data sets, except plasma PTH, plasma calcitriol, urine pH and urine Cl concentration, presented normal distribution. For normally distributed data, comparisons between diets and at different times were carried out with the paired Student *t* test. Mann-Whitney analysis followed by a post hoc rank total test was used for plasma PTH, plasma calcitriol, urine pH and urine Cl concentration. Correlation was studied with the Pearson test. A *P* value less than 0.05 was considered significant. Results are

Table 2
Parameters of mineral metabolism in plasma samples obtained from cats before (0 days) and after (30 and 60 days) being fed calculolytic diets (Diet 1 and Diet 2).

		Time (days)		
		0	30	60
	Diet	Mean ± SE	Mean ± SE	Mean ± SE
iCa (mmol/L)	1	1.3 ± 0.01	1.3 ± 0.01	1.3 ± 0.01
	2	1.3 ± 0.01	1.3 ± 0.01	1.3 ± 0.01
tCa (mg/dL)	1	9.9 ± 0.4	9.9 ± 0.2	9.8 ± 0.2
	2	9.8 ± 0.3	9.7 ± 0.3	10.7 ± 0.6
P (mg/dL)	1	5.1 ± 0.3	4.7 ± 0.2	4.6 ± 0.3
	2	5.0 ± 0.3	5.1 ± 0.4	4.9 ± 0.3
Mg (mg/dL)	1	2.3 ± 0.1	2.3 ± 0.1	2.1 ± 0.1
	2	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
PTH (pg/mL)	1	8.9 ± 2.0	7.8 ± 1.7	5.6 ± 1.5
	2	7.8 ± 1.6	7.8 ± 1.7	7.0 ± 1.5
Calcitriol (pg/mL)	1	111.0 ± 11.7	96.0[a] ± 12.0	91.1[a] ± 11.6
	2	110.4 ± 12.5	84.8[a] ± 9.8	82.5[a] ± 9.6

Diet 1, Prescription Diet Feline s/d, Hill's, Madrid, Spain; Diet 2, Veterinary Diet Urinary S/O Feline, Royal Canin, Aimargues, France; iCa, ionized calcium; tCa, total calcium; P, phosphorus; Mg, magnesium; PTH, parathyroid hormone.
Comparison between times: [a] $P>0.05$ vs time 0.

expressed as the mean ± standard error.

3. Results

Changes in the parameters under study after feeding calculolytic diets were compared with baseline values obtained when cats were fed the standard diet. Plasma creatinine concentration was unaffected with Diet 1 and tended to decrease with Diet 2 (from 1.2 ± 0.1 to 1.1 ± 0.1 mg/dL, at 30 days, $P<0.05$).

Feeding on the calculolytic diets for two months did not alter plasma concentrations of iCa, tCa, P, Mg and PTH. However, a marked decline in calcitriol was observed after administering both treatment diets: from 111.0 ± 11.7 to 91.1 ± 11.6 pg/mL (Diet 1) and from 110.4 ± 12.5 to 82.5 ± 9.6 pg/mL (Diet 2) $P<0.05$ (Table 2). No changes in plasma K and Cl were found after feeding the calculolytic diets. A tendency to decreased plasma Na, which was significant with Diet 2 at 30 days (from 157.3 ± 1.1 to 153.6 ± 0.7 mmol/L, $P<0.05$) was identified.

Both diets decreased urine specific gravity and the decrease was more accentuated at 30 days, from 1069 ± 2 to 1061 ± 3 g/mL (Diet 1) and from 1065 ± 4 to 1051 ± 2 g/mL (Diet 2). Changes in urine pH were only observed after feeding Diet 1 (from 6.2 ± 0.3 to 5.7 ± 0.1, at 60 days, $P<0.05$). Cats fed Diet 1 showed a significant increase in urine Ca concentration (from 1.3 ± 0.1 to 1.9 ± 0.1 mg/dL) and FECa (from 0.06 ± 0.01 to 0.08 ± 0.01%) at 60 days.

Table 3
Minerals in urine samples obtained from cats before (0 days) and after (30 and 60 days) being fed calculolytic diets (Diet 1 and Diet 2).

		Time (days)		
		0	30	60
	Diet	Mean ± SE	Mean ± SE	Mean ± SE
UCa (mg/dL)	1	1.3 ± 0.1	1.4 ± 0.1	1.9[a,b,*] ± 0.1
	2	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
FECa (%)	1	0.06 ± 0.006	0.06 ± 0.003	0.08[a,b,*] ± 0.006
	2	0.06 ± 0.006	0.06 ± 0.008	0.05 ± 0.005
UP (mg/dL)	1	281.4 ± 18.1	200.8[a,*] ± 17.7	270.6[b,*] ± 23.8
	2	235.5 ± 25.0	139.1[a] ± 11.2	167.4[a] ± 16.1
FEP (%)	1	26.4 ± 2.4	17.6[a] ± 2.0	24.4[b,*] ± 2.4
	2	22.6 ± 3.2	15.9 ± 2.5	16.4 ± 1.3
UMg (mg/dL)	1	2.8 ± 0.3	4.3[a] ± 0.3	4.0[a] ± 0.2
	2	2.7 ± 0.3	4.1[a] ± 0.4	4.4[a] ± 0.6
FEMg (%)	1	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
	2	0.6 ± 0.1	1.0[a] ± 0.2	1.0 ± 0.2

Diet 1, Prescription Diet Feline s/d, Hill's, Madrid, Spain; Diet 2, Veterinary Diet Urinary S/O Feline, Royal Canin, Aimargues, France; UCa, urine calcium; FECa, fractional excretion of calcium; UP, urine phosphorus; FEP, fractional excretion of phosphorus; UMg, urine magnesium; FEMg, fractional excretion of magnesium.
Comparison between times: [a] $P>0.05$ vs time 0; [b] $P>0.05$ vs time 30. Comparison between diets: * $P>0.05$ vs Diet 2.

Urine P concentration was decreased 30 days after feeding both diets and tended to recover at 60 days. Fractional excretion of P only decreased after feeding Diet 1 for 30 days (from 26.4 ± 2.4 to 17.6 ± 2.0%). Magnesium concentration in urine was increased with both diets, from 2.8 ± 0.3 to 4.0 ± 0.2 mg/dL (Diet 1) and from 2.7 ± 0.3 to 4.4 ± 0.6 mg/dL (Diet 2). However, significant differences in FEMg were only found after feeding Diet 2 for 30 days (Table 3). Fractional excretions of Na, and Cl remained unaffected in cats receiving Diet 1 and were increased ($P<0.05$) 60 days after feeding Diet 2 (FENa: 0.88 ± 0.06 vs 0.26 ± 0.05%; FECl: 1.20 ± 0.09 vs 0.71 ± 0.17%).

A strong correlation between FENa and FEMg was observed when cats were fed Diet 2 (r=0.795, P=0.001) but not when they received Diet 1 (r=-0.182, P=0.571) (Figure 1). An inverse correlation between urine pH and FEMg (r= -0.376, P=0.001) and FECa (r= -0.277, P=0.018) was measured after feeding both diets.

4. Discussion and conclusions

As would be expected, all diets used in the study provided the basic nutrient requirements for cats (NRC, 2006). The calculolytic diets had lower mineral content (lower Ca, P and Mg) and lower vitamin D concentration than the standard diet. However, no major changes in the plasma concentrations of iCa, tCa, P and Mg were found after feeding the

calculolytic diets. Thus, the reduced Ca, P and Mg content of these diets seems to be sufficient to maintain normal plasma levels of these macroelements. The finely tuned homeostatic mechanisms (mainly PTH secretion which regulates bone resorption and urinary excretion) maintain plasma Ca, Mg and P concentrations quite constant and changes in these minerals are usually found after receiving an inappropriate supply over a long period. Although changes in plasma mineral concentration would likely be undetectable, an inadequate calcium supply would be reflected by an increase in plasma PTH. Since no changes in plasma PTH were detected it would be reasonable to conclude that the low mineral concentrations in calculolytic diets are adequate to provide basic needs.

The lower vitamin D content of the calculolytic diets (when compared with the standard diet) resulted in a decrease in the plasma concentration of calcitriol, the major vitamin D metabolite. The significance of these reduced calcitriol levels is questionable. The concentrations of calcitriol measured after feeding calculolytic diets are within normal reference ranges (Pineda et al., 2013) and did not affect plasma Ca concentrations. Moreover, as noted above, the lower calcitriol concentrations were not accompanied by any increase in PTH. Current recommendations for vitamin D intake in cats are 6.25 μg/Kg (NRC, 2006), however the exact vitamin D requirements for cats remain unclear. In other species vitamin D requirements have been established by determining the point at which a decrease in vitamin D intake gives rise to an increase in plasma PTH concentrations (IOM, 2011). Following this criterion vitamin D concentrations provided by the present calculolytic diets seem to be sufficient to meet normal requirements in healthy cats. In fact, the concentrations of vitamin D in calculolytic diets could be more appropriate for cats than the high concentrations normally found in standard diets. This contention is not new, as it has been suggested that feline standard diets are oversupplemented with vitamin D and this may lead to dental problems and hypercalcemia in some cats (Midkiff et al., 2000; Reiter et al., 2005).

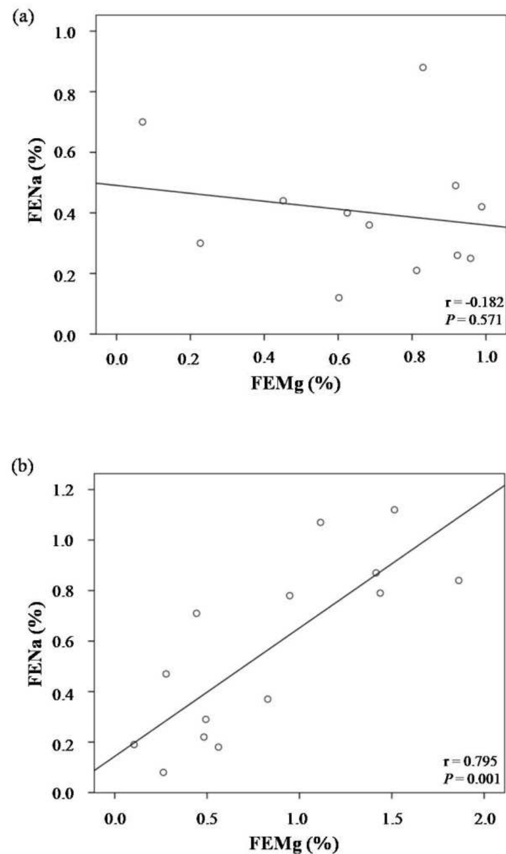
Both calculolytic diets decreased urine specific gravity. This effect was more accentuated with Diet 2 and was likely due to its higher salt content. The decrease in urine specific gravity was accompanied by a significant decrease in plasma creatinine concentration in cats receiving Diet 2, suggesting a diuretic effect

of the dietary salt which would be related to increased water intake. The impact of dietary NaCl on water intake in cats has been experimentally evaluated.

Anderson (1982) reported that cats fed diets with low (1.4% DM) NaCl had lower water intake when compared with those fed a high (4.6% DM) NaCl diet (130 vs 250 mL water/day). Additionally, a diet with elevated Na content has been reported to increase urine volume and decrease both urine specific gravity and urine calcium oxalate relative supersaturation (Hawthorne and Markwell, 2004). In humans, high Na intake has been related to the development of hypertension and osteoporosis (Heaney, 2006; Woo et al., 2009). However, in healthy cats feeding diets high in Na does not seem to have deleterious consequences in terms of blood pressure and bone mineral content (Xu et al., 2009; Reynolds et al., 2013). Thus, increasing salt intake may be a viable option to promote diuresis in cats; however, the tolerance to diets with high NaCl content may be reduced in cats with compromised renal function, a common scenario in cats requiring calculolytic diets (Kerr, 2013).

Urine pH was significantly decreased when cats were fed Diet 1 but not when they were fed Diet 2. The reduction in urine pH may have influenced the increased urinary excretion of Ca and Mg in

Figure 1 – Correlation between fractional urinary excretion of sodium (FENa) and fractional urinary excretion of magnesium (FEMg) in cats fed Diet 1 (a) and Diet 2 (b).



cats fed Diet 1, as shown by the correlation study and supported by previous data in the literature (Ching et al., 1989). In other species, dietary changes leading to metabolic acidosis may increase glomerular filtration of Ca, decrease renal tubular reabsorption of Ca and promote skeletal mobilization of Ca (Zerwekh and Pak, 1982). In adult cats, dietary acidification has been

reported to cause hypercalciuria (Ching et al., 1989; Ching et al., 1990) and hypermagnesiuria (Houillier et al., 1996). The increase in urinary Ca and Mg seems to be mediated by a decrease in tubular Ca and Mg reabsorption, independent of PTH, but dependent on changes in net acid excretion. The available data suggest that acid load influences the reabsorption of divalent cations at the thick ascending limb of Henle's loop (Houillier et al., 1996).

As expected by the low P content of the diets, urine P concentration and FEP tended to decrease compared to feeding the standard diet with a higher P concentration. It is interesting to note that the decrease was more marked on day 30 of the study compared to day 60 of the study. Thus, it seems that the kidney adapts to the lower P intake and tends to stabilize FEP with time. Therefore, the decrease in urine P concentration, that would help to prevent struvite crystal formation, may be transitory.

An interesting finding of this work was the increase in urine Mg concentration and FEMg after feeding the calculolytic diets, an effect that was more evident with Diet 2. This increase in urinary Mg seems both paradoxical, since the diets, especially Diet 2, have lower Mg concentrations compared to standard diets, and counterproductive to prevent struvite crystal formation. The increase in urine Mg excretion after feeding Diet 1 is likely influenced by the decrease in urine pH, as discussed above. In cats receiving Diet 2, the increase in Mg excretion by urine seems to be associated to the high Na content of the diet and the subsequent increase in urinary Na excretion. In experimental rodent models and in humans an increase in salt intake has been shown to increase urinary Ca and Mg loss (Chan et al., 1992; Massey and Whiting, 1996; Lee et al., 2012) suggesting that after modification of dietary salt and water intake the kidney adapts with corresponding changes in divalent cation excretion. In our study, correlation between Na and Mg excretion was excellent when the diet was high in Na but non-existent when Na content was normal. Thus, our results support the influence of Na intake on divalent cation excretion by urine in cats, although in the cats of the present study the effect was mostly seen in Mg and, to a lower extent, in Ca.

In conclusion, contrary to our hypothesis, the calculolytic diets did not seem to negatively affect mineral metabolism. Although a decrease in plasma calcitriol levels, related to the lower vitamin D content of calculolytic diets, has been found, the clinical significance of this finding is

questionable. Both diets resulted in an increased urinary excretion of Mg through different mechanisms: urine acidification and increased Na load.

Conflict of interest statement: None of the authors of this paper has any
financial or personal relationship with other people or organizations which might inappropriately influence or bias the content of the paper.

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References

Anderson, R.S., 1982. Water balance in the dog and cat. Journal of Small Animal Practice 23, 588-598.

Chan, E.L., Ho, C.S., MacDonald, D., Ho, S.C., Chan, T.Y., Swaminathan, R., 1992. Interrelationships between urinary sodium, calcium, hydroxyproline and serum PTH in healthy subjects. Acta Endocrinologica 127, 242-245.

Ching, S.V., Fettman, M.J., Hamar, D.W., Nagode, L.A., Smith, K.R., 1989. The effect of chronic dietary acidification using ammonium chloride on acid-base and mineral metabolism in the adult cat. The Journal of Nutrition 119, 902-915.

Ching, S.V., Norrdin, R.W., Fettman, M.J., LeCouteur, R.A., 1990. Trabecular bone remodeling and bone mineral density in the adult cat during chronic dietary acidification with ammonium chloride. Journal of Bone and Mineral Research 5, 547-556.

Hawthorne, A.J., Markwell, P.J., 2004. Dietary sodium promotes increased water intake and urine volume in cats. The Journal of Nutrition 134, 2128S-2129S.

Heaney, P.R, 2006. Role of dietary sodium in osteoporosis. Journal of the American College of Nutrition 25, 271S-276S.

Houillier, P., Normand, M., Froissart, M., Blanchard, A., Jungers, P., Paillard, M., 1996. Calciuric response to an acute acid load in healthy subjects and hypercalciuric calcium stone formers. Kidney International 50, 987-997.

Institute of Medicine (IOM), 2011. Dietary reference intakes for calcium and vitamin D. The National Academies Press (USA), Washington, DC.

Kerr, K.R., 2013. Companion Animals Symposium: dietary management of feline lower urinary tract symptoms.

Journal of Animal Science 91, 2965-2975.
Kirk, C.A., Jewell, D.E., Lowry, S.R., 2006. Effects of sodium chloride on selected parameters in cats. Veterinary Therapeutics 7, 333-346.
Lee, C.T., Lien, Y.H., Lai, L.W., Ng, H.Y., Chiou, T.T., Chen, H.C., 2012. Variations of dietary salt and fluid modulate calcium and magnesium transport in the renal distal tubule. Nephron Physiology 122, 19-27.
Massey, L.K., Whiting, S.J., 1996. Dietary salt, urinary calcium, and bone loss. Journal of Bone and Mineral Research 11, 731-736.
Midkiff, A.M., Chew, D.J., Randolph, J.F., Center, S.A., DiBartola, S.P., 2000. Idiopathic hypercalcemia in cats. Journal of Veterinary Internal Medicine 14, 619-626.
National Research Council, 2006. Nutrient Requirements of dogs and cats. The National Academies Press (USA), Washington, DC, pp. 204-205.
Pineda, C., Aguilera-Tejero, E., Guerrero, F., Raya, A.I., Rodriguez, M., Lopez, I., 2013. Mineral metabolism in growing cats: changes in the values of blood parameters with age. Journal of Feline Medicine and Surgery 15, 866-871.
Pineda, C., Aguilera-Tejero, E., Raya, A.I., Diez, E., Rodriguez, M., Lopez, I., 2012. Feline parathyroid hormone: validation of hormonal assays and dynamics of secretion. Domestic Animal Endocrinology 42, 256-264.
Reiter, A.M., Lewis, J.R., Okuda, A. 2005. Update on the etiology of tooth resorption in domestic cats. The Veterinary Clinics of North America. Small Animal Practice 35, 913-942.
Reynolds, B.S., Chetboul, V., Nguyen, P., Testault, I., Concordet, D.V., Carlos Sampedrano, C., Elliott, J., Trehiou-Sechi, E., Abadie, J., Biourge, V., Lefebvre, H.P., 2013. Effects of dietary salt intake on renal function: a 2-year study in healthy aged cats. Journal of Veterinary Internal Medicine 27, 507-515.
Woo, J., Kwok, T., Leung, J., Tang, N., 2009. Dietary intake, blood pressure and osteoporosis. Journal of Human Hypertension 23, 451-455.
Xu, H., Laflamme, D.P., Long, G.L., 2009. Effects of dietary sodium chloride on health parameters in mature cats. Journal of Feline Medicine and Surgery 11, 435-441.
Zerwekh, J.E., Pak, C.Y., 1982. Mechanisms of hypercalciuria. Pathobiology Annual 12, 185-201.

Impact factor and quartile report from the Journal Citation Reports (SCI and/or SSCI) or from the database reference area in which the scientific articles have been published

Informe con el factor de impacto y cuartil del Journal Citation Reports (SCI y/o SSCI) o de las bases de datos de referencia del área en el que se encuentran las publicaciones presentadas

Pineda C, Aguilera-Tejero E, Raya AI, Diez E, Rodriguez M, Lopez I. Feline parathyroid hormone: validation of hormonal assays and dynamics of secretion. *Domest Anim Endocrinol* 2012; 42: 256-264.

Pineda C, Aguilera-Tejero E, Guerrero F, Raya AI, Rodriguez M, Lopez I. Mineral metabolism in growing cats: changes in the values of blood parameters with age. *J Feline Med Surg* 2013; 15: 866-871.

Pineda C, Aguilera-Tejero E, Raya AI, Guerrero F, Rodriguez M, Lopez I. Assessment of calcitonin response to experimentally induced hypercalcemia in cats. *Am J Vet Res* 2013; 74: 1514-1521.

Pineda C, Aguilera-Tejero E, Raya AI, Montes de Oca A, Rodriguez M, Lopez I. Effects of calculolytic diets on feline mineral metabolism. *In Press*.

Abbreviated Journal Title *(linked to journal information)*	ISSN	JCR Data					
		Total Cites	Impact Factor *(for 2012)*	5-Year Impact Factor	Immediacy Index	Articles	Cited Half-life
DOMEST ANIM ENDOCRINOL	0739-7240	1927	2.377	2.322	0.207	58	7.8
J FELINE MED SURG	1098-612X	1310	1.080	1.446	0.154	130	4.4
AM J VET RES	0002-9645	9392	1.348	1.614	0.193	254	>10.0

Journal Title	Category Name	Total Journals in Category	Journal Rank in Category	Quartile in Category
DOMEST ANIM ENDOCRINOL	AGRICULTURE, DAIRY & ANIMAL SCIENCE	54	4	Q1
	ENDOCRINOLOGY & METABOLISM	122	67	Q3
J FELINE MED SURG	VETERINARY SCIENCES	143	61	Q2
AM J VET RES	VETERINARY SCIENCES	143	44	Q2

DISCUSSION

The discussion of the work that has been done in this Doctoral Thesis will be divided by objectives:

1. Validation of commercially available human I-PTH and W-PTH assays for measurement of feline PTH

In domestic animals measurements of certain endocrine parameters are often difficult to make by the lack of assays using specific antibodies for each species. It has been demonstrated that the PTH molecule is similar in different species.[11,109] For that reason, heterologous assays can be used reliably to measure PTH in mammals. In fact, human PTH assays have been successfully validated for measurement in domestic animals.[5,33,112]

In the present work two PTH assays (I-PTH and W-PTH) have been validated by assessment of their accuracy, precision, sensitivity, reproducibility, and also by evaluating changes in PTH after modifying plasma calcium concentrations.

In a previous study Barber et al.[5] validated a two-site human IRMA for measurement of feline PTH but this assay is currently not available. In the present Doctoral Thesis, a commercially available assay for measurement of I-PTH and W-PTH has been validated for measurement of feline PTH. Values of precision (9.8% to 11.1%) and sensitivity (2 pg/mL) were found to be somewhat better in our study when compared with the data obtained by Barber et al.[5] (precision 12.7%; sensitivity 3.9 pg/mL). Furthermore, Williams et al.[118] have recently validated the same I-PTH assay that we used in our study. Similarly when compared our results with this work (precision 18.9%; sensitivity 5.2 pg/mL) we have obtained slightly better values.

On the other hand, both assays (I-PTH and W-PTH) had similar intra-assay precision. However, the interassay coefficients of variability were

slightly greater when measuring W-PTH. This finding contrasts with the work of Estepa et al.,[33] who observed a better reproducibility for the W-PTH than for I-PTH in equine samples. It is improbable that species differences explain this discrepancy. Variability in the assay production and/or sample handling seems more likely explanations.

In the present work the I-PTH levels in healthy cats were 9.1 ± 0.7 pg/mL. This value was similar to the data reported by Barber et al.[5] (10.9 ± 5.3 pg/mL) (mean ± standard deviation). However, the data obtained by Williams et al.[118] was slightly higher (13.8 pg/mL). These higher values may be explained by the age of the animals, because the study of Williams et al.[120] was performed in a group of geriatric cats (older than nine years). Parathyroid hormone has previously been reported to be elevated in aged patients, suggesting that the increase in PTH with ageing may be a common finding in elderly animals.[2]

One interesting and somewhat unexpected finding found in our study was that greater PTH values were obtained when measuring with the W-PTH assay than when using the I-PTH assay. Although it is an unusual finding, the same phenomenon was found by our research group in a previous study of equine PTH.[35] The greater W-PTH than I-PTH values may be related to different affinity of the W-PTH and I-PTH tracer antibody against feline PTH. Thus, the tracer antibody against human 7-34 PTH, used by the I-PTH assay, would have lower affinity for the feline PTH molecule than the tracer antibody against 1-4 PTH, used in the W-PTH assay. If this were the case, the I-PTH assay would be detecting both 1-84 PTH and N-terminal truncated fragments, but its ability to bind the 7-34 region would be inferior to the binding capacity of the antibody against the 1-4 region and, consequently, the PTH concentration would be lower when measured with I-PTH than when measured with W-PTH. This hypothesis seems reasonable because the part of the PTH molecule that shows less interspecies variation is the amino-terminal end.[84] In addition, feline PTH

has been reported to be similar to human PTH in the amino-terminal portion[109]; therefore, heterologous antibodies should be more likely to bind the most amino-terminal part of the PTH molecule.

Our study has demonstrated that both assays were able to detect changes in PTH when plasma calcium was modified in the study of the PTH-Ca curve (increase in PTH during hypocalcemia and decrease in PTH during hypercalcemia).

It is important to note that both assays can be used to measure PTH concentrations in feline samples and both assays have clinical utility. Intact PTH is more widely used in human and in veterinary medicine and is more available for the measurement of individual samples. However, W-PTH values should reflect more accurately PTH activity than I-PTH values.

In conclusion, the results of our study demonstrated that the I-PTH and W-PTH assays were capable to measure feline PTH. Both assays showed good accuracy, precision, sensitivity, reproducibility, and ability to detect changes in PTH in response to changes in calcium.

2. Dynamics of PTH secretion in response to changes in extracellular calcium in healthy cats

To assess the PTH response to changes in plasma calcium concentrations, hypo- and hypercalcemia were induced in a group of healthy cats. It is known that the PTH response to changes in calcium is best represented by the PTH-Ca curve, which is obtained by plotting PTH against calcium in a range of calcium concentrations.[15]

Dynamics of PTH secretion were evaluated with both PTH assays (I-PTH and W-PTH). The shape of the PTH-Ca curve obtained with the two

assays was practically identical, which reinforces the concept that both assays are suitable for measurement of feline PTH.

When evaluating the variables derived from the PTH-Ca curve, basal and maximal W-PTH were greater than the corresponding values of I-PTH. However, minimal W-PTH and I-PTH were similar. The differences between W-PTH and I-PTH along the PTH-Ca curve, which could be explained by secretion of different PTH fragments at different calcium concentrations, were best observed when studying the W-PTH/I-PTH ratio. This ratio increased in response to hypocalcemia (when more "active" W-PTH is needed) and decreased during hypercalcemia (when less "active" W-PTH is required). This finding has been previously reported by our laboratory in horses.[35] However, in dogs we were unable to find significant changes in the ratio during hypocalcemia.[36]

The feline PTH-Ca curve showed notable similitude to the canine PTH-Ca curve[1,34,96] especially when the W-PTH values were considered. Basal, maximal, and minimal W-PTH values in cats were similar to the previously reported values in dogs. This is important because the dog has been extensively used as an animal model for the study of PTH and thus much of the information gathered in dogs could be substantially extrapolated to cats. Feline PTH-Ca curve also shared great similarity to the curve reported in humans.[39]

In conclusion, the PTH-Ca curve in cats was similar to the canine curve and thus the extensive knowledge on dynamics of PTH secretion in dogs that can be found in the literature could be extrapolated to cats.

3. Changes in the main parameters involved in mineral metabolism (calcium, phosphorus, magnesium, PTH, calcitriol, and calcidiol) during the growing process in healthy cats aged 3 to 15 months

Concentrations of blood parameters related to mineral metabolism change along the growing process of the cat. Previous studies have reported that kittens younger than 8 weeks have greater plasma calcium than adults.[65] Our results showed that between 3 and 12 months of age tCa concentration was significantly higher than values measured at 15 months. Moreover, the biologically active form of calcium, iCa, which to our knowledge, has not been studied in growing kittens, showed a steady and progressive decrease during the first year of life and stabilized between 12 and 15 months of age. The difference in the reduction of iCa and tCa at younger ages may be explained by changes in plasma albumin. Lower albumin concentrations, as measured in younger cats,[65] will result in lesser tCa values and thus may explain why tCa was proportionally lower than iCa at these young ages.

In the present study, a decrease in both plasma phosphorus and magnesium concentrations, which followed the same time-frame than the decline in tCa, has been identified. Previous studies have also reported that immature cats tend to have higher serum phosphorus concentrations.[65] However, age variations related to magnesium concentrations in kittens had not been reported previously.[63]

In relation to mineral metabolism hormones, plasma PTH concentrations (I-PTH and W-PTH) remained stable during the study period with only a small increase around 1 year of age, which does not seem to be relevant for diagnostic purposes. The only reference to PTH values in growing cats that we have been able to find was provided by Tomsa et al.[108] who measured I-PTH in seven healthy cats (4-6 months old) and used then as control values to compare with cases of nutritional

secondary hyperparathyroidism. The I-PTH values reported by Tomsa et al.[108] (<3-28 pg/mL) are comparable with the I-PTH concentrations measured in our study.

As we described in the previous study of PTH in adult cats,[83] W-PTH concentrations were also higher than I-PTH concentrations in growing kittens. We have reported an increase in the W-PTH/I-PTH ratio in hypocalcemic adult cats, supporting the notion that this ratio tends to increase when more "active" PTH is needed.[83] Although these ratios should be interpreted with caution when using heterologous assays, the decline in the W-PTH/I-PTH ratio with age detected in kittens would be consistent with a decrease in the need for "active" PTH once the skeletal growth is completed.

In the present study fluctuations in calcitriol and calcidiol concentrations have been detected during the growth process. These changes may be explained by the vitamin D requirements during this stage. One possible explanation for this finding, based on previous studies about vitamin D metabolites in other species,[60,76,105] is that at younger ages the pro-vitamin D, calcidiol, is lower because it is being used to produce calcitriol, and calcitriol is higher to facilitate skeletal growth. When the growing needs decrease calcitriol concentration also declines and, owing to reduced demand, calcidiol increases.

The quantification of the vitamin D metabolites is essential in cases in which disturbances in mineral metabolism are suspected, especially in patients with bone disease. In the literature, vitamin D-dependent rickets has been described in kittens. In these cases evaluation of calcitriol and calcidiol concentrations is clinically relevant for the diagnosis.[31,44,45,53,68,82,103,107] Two major subtypes of vitamin D-dependent rickets have been reported in kittens: type I and type II. Type I is caused by a defect in the gene encoding the enzyme 1-alphahydroxylase that

converts calcidiol to calcitriol,[44] while type II is associated with mutations in the gene encoding the vitamin D receptor (VDR).[45,68,103,107] In reported type I cases, levels of calcidiol are increased but failure of the hydroxylation in the renal tubules leads to low levels of calcitriol. In type II cases, levels of calcidiol are either normal or decreased but levels of calcitriol are increased due to “receptor resistance”.[31]

Given that the diagnosis of feline vitamin D-dependent rickets is based in the evaluation of calcitriol and calcidiol plasma concentrations, it is evident that to provide an accurate interpretation of vitamin D status reference values adequate to the age of the kitten are needed. However, to our knowledge, these reference intervals have not been available before and most authors have likely used adult reference ranges. Our results seem to indicate that calcitriol values in growing kittens were higher than previously thought.

Reference intervals for calcidiol used previously in the diagnosis of rickets are comparable to our values. Our data were quite similar to the results reported by Morris et al.,[75] who investigated the effect of dietary vitamin D content on plasma calcidiol concentrations. They also found a tendency of increasing calcidiol with age that was more pronounced when diets with abnormally high vitamin D content were fed.

Thus, a major contribution of the present work is to provide values for calcitriol and calcidiol in growing kittens at different stages of skeletal maturity. In addition, given the fact that the concentrations of these metabolites are associated with growth, when used for diagnostic purposes, values should be compared with the range according to the age of the kitten.

In conclusion, from 3 months to 15 months of age, cats showed a decrease in plasma minerals: calcium (both tCa and iCa), phosphorus, and

magnesium. No major changes in PTH (both I-PTH and W-PTH) were evident, although the W-PTH/I-PTH ratio decreased significantly with age. A reciprocal change in vitamin D metabolites (decrease in calcitriol and increase in calcidiol) has been identified during the growing process. Moreover, the adult levels of all parameters under study were within the reference ranges of our laboratory for feline samples.

4. Validation of a commercially available human CT assay for quantification of feline CT and providing normal values in healthy cats

Disturbances of calcium metabolism (eg, hypercalcemia of malignancy and hypo- or hypercalcemia associated with renal failure and nutritional disorders) are common in cats.[7,98] Moreover, cats can develop unexplained hypercalcemia, such as idiopathic hypercalcemia, with unknown etiopathogenesis.[73] To have a comprehensive understanding of these disorders, it is necessary to know the dynamics of secretion of calciotropic hormones.

In cats as well as in most domestic animals, endocrine studies are often complicated by the lack of assays with specific antibodies. It should be noted that CT molecules are quite similar among different species.[74] Thus, heterologous assays can be used to reliably measure CT concentration in some other mammals.

Compared with PTH, information about CT in domestic animals is much more incomplete. Although a specific assay for quantification of canine CT has been described[49] and measurement of plasma CT levels has been recently reported in horses,[95] no data are available in cats to our knowledge.

In our study, the CT assay was validated for use in cats by assessment of its precision, specificity, and sensitivity, and also by evaluating CT concentrations in plasma samples with different levels of calcium.

In feline plasma samples, the CT assay had a limit of detection of 0.9 pg/mL. The assay had an intra-assay coefficient of variation of 12.8% for feline samples with apparently normal plasma CT concentration (ie, samples from normocalcemic cats) and 4.5% for feline samples with high plasma CT concentration (ie, samples from hypercalcemic cats). Interassay coefficient of variation ranged from 18.6% to 13.3%. Specificity was assessed by dilutional parallelism. A predictable dilution pattern was observed in samples with high CT concentration. The assay demonstrated adequate dilutional parallelism with percentages of recovery ranging from 124% to 129%.

Our results showed very low CT concentrations in clinically healthy cats. It must be taken into consideration that it is not uncommon to find low CT concentrations in other species. In fact, healthy humans may have basal CT concentrations similar to what we have found in cats (0.9 to 3.2 pg/mL) and nearly undetectable CT concentrations are considered normal in humans.[87]

In conclusion, the results of our study demonstrated that CT assay can be used for measurement of feline CT. The assay showed good precision, specificity, and sensitivity.

5. Dynamics of CT secretion in response to experimentally induced hypercalcemia and the related changes in extracellular iCa concentration in healthy cats

In domestic animals, the CT response to changes in calcium concentration has been studied in dogs and horses that received an intravenous bolus of calcium.[49,95] Compared to cats, basal and maximum CT concentrations seemed to be higher both in dogs[49] and horses.[95] Although previous studies[49,95] provide evidence of CT response to changes in calcium concentration in domestic animals, those data do not allow a thorough evaluation of the CT-Ca curve. Thus, we had to compare results of the present study with data from rats, the only animal species for which a detailed CT-Ca curve has been described.[113,115,116] The shape and the set point of the curve for cats and rats were similar. However, basal and maximum CT concentrations were much higher in rats than in cats. In addition, the heterogeneity in the CT response to hypercalcemia that we found in our group of cats has not been reported in other mammals, to our knowledge.

The significance of CT in the control of mineral metabolism is quite different between species. It is known that, in rats, CT seems to be very important for avoiding development of hypercalcemia. Results of one study[116] have indicated a tendency for rats to become hypercalcemic after thyroparathyroidectomy or after selective thyroidectomy. By contrast, in thyroidectomized cats, hypocalcemia is reportedly the main complication; hypocalcemia that develops in some cats after total thyroidectomy is assumed to be related to damage to the parathyroid glands.[77] Nonetheless, the fact that the ablation of thyroid gland tissue does not result in hypercalcemia suggests that CT has less influence than PTH on calcium metabolism in cats. Taken together, the results of the present study (low basal CT concentration plus moderate and heterogeneous response to

hypercalcemia) would support the contention that CT seems to have a secondary role in feline calcium homeostasis.

One of the more striking findings of the present study was the fact that some cats did not have an increase in plasma CT concentration in response to experimentally induced hypercalcemia. This surprising discovery was confirmed through repeated experiments. Given that plasma CT concentrations were not increased at any time in nonresponder cats, the differences between the responders and nonresponders should be at the level of secretion rather than at the level of clearance. No significant differences were found in basal CT concentration between responders and nonresponders; however, considering that basal CT was very low in both subgroups, it would be unlikely to find such differences.

When analyzing the iCa concentration changes as a result of the calcium chloride infusion, it was interesting to note that there was no difference between the two subgroups of cats. The rate of calcium changes, which has been shown to influence CT secretion,[115] was almost identical in both responders and nonresponders. Thus, the absence of CT secretion did not result in a more profound hypercalcemia in nonresponders.

To determine whether the lack of CT would be compensated by changes in PTH concentration, final PTH concentration at the end of hypercalcemia and the difference in PTH concentration (initial minus final concentrations) in both subgroups were compared. Again, no significant difference in either variable was found between responders and nonresponders on the basis of data obtained with I-PTH or W-PTH assays. Also, no differences in vitamin D status or in thyroid gland function were found between the two subgroups of cats. Immunohistochemical analysis of thyroid gland tissue revealed that the density of cells expressing CT was higher in responders than in nonresponders. Thus, a histologic basis to

explain the disparate response to hypercalcemia between the two subgroups of cats was found.

In humans, gender is known to affect the calcium response of CT. Men have higher basal CT concentrations and a more pronounced CT response to hypercalcemia than women.[52] It is interesting to note that men have also been reported to have twice as many C cells as women.[47] In our study, the lack of CT response to hypercalcemia was not related to sex, given that both males and females were included among the nonresponders. However, we believe that the disparity in the number of CT-positive C cells found between the subgroups of cats and its correlation with CT response to hypercalcemia is the basis of the heterogeneity in CT secretion found in the present study.

Although the data obtained in the present study seem to support a minor role of CT in the control of iCa concentration in cats, it must be noted that we evaluated healthy cats that were induced to develop acute hypercalcemia and that the situation may be different in cats with chronic hypercalcemia. Since 1990, unexplained hypercalcemia has been increasingly noted as an incidental finding on serum biochemistry profiles of cats and its pathophysiology remains unexplained.[73] Based on the findings of the present study, it could be rewarding to investigate plasma CT concentrations in cats with idiopathic hypercalcemia. In a chronic situation, nonresponders may have limited capacity to counteract hypercalcemia, not only because of the lack of direct hypocalcemic effect of CT but also because CT is known to decrease the calcemic response to PTH; thus, in the absence of CT, PTH would be expected to promote hypercalcemia.[90]

In conclusion, the results of the present study demonstrated that CT assay was capable of detecting changes in CT response to changes in calcium. Moreover, the results indicated a heterogeneous CT response to

hypercalcemia in cats. Two subgroups of cats were distinguished: cats in which plasma CT concentration increased during hypercalcemia and cats in which CT concentration remained very low even at very high iCa concentrations that are consistent with clinical hypercalcemia in cats. Although no deleterious effects in the acute response to hypercalcemia were detected in the nonresponders, cats in which plasma CT concentration did not increase could be predisposed to disorders of calcium metabolism.

6. To evaluate the influence of two calculolytic diets on parameters of mineral metabolism in cats

Nutritional management, through the use of calculolytic diets, is quite effective in preventing and treating struvite uroliths in cats.[61] Since these diets are fed for prolonged periods (months to years) they are potentially able to modify mineral metabolism.[20]

Compared with standard diets, the calculolytic diets used in this study have lower mineral content and lower vitamin D concentration. However, in this work, no major changes in the plasma levels of iCa, tCa, phosphorus, and magnesium were found after feeding the calculolytic diets. Although plasma mineral concentration can be maintained by increasing bone resorption, the lack of increase in PTH concentrations seems to indicate that in the cats under study an increase in bone resorption was not required to maintain the plasma concentration of minerals within normal ranges.

The lower vitamin D content of the calculolytic diets (compared with the standard diet) resulted in a decrease in the plasma level of calcitriol, the major vitamin D metabolite. The significance of these reduced calcitriol concentrations is questionable. The concentration of calcitriol measured

after feeding calculolytic diets did not seem to impair calcium metabolism as reflected by normal calcium concentration. In addition, the lower level of calcitriol was not accompanied by any increase in PTH. Current recommendations for vitamin D intake in cats are 6.25 μg/kg,[78] however the exact vitamin D requirements for cats remain unclear. In humans, vitamin D requirements have been established by determining the point at which a decrease in vitamin D intake gives rise to an increase in PTH concentrations.[58] Following this criterion, the vitamin D concentration provided by calculolytic diets are sufficient to meet normal requirements in healthy cats. In fact, the concentrations of vitamin D in calculolytic diets could be more appropriate for cats that the high concentrations normally found in standard diets. This contention is not new, as it has been suggested that feline diets are oversupplemented with vitamin D and this may lead to dental problems and hypercalcemia in some cats.[73,88]

Both calculolytic diets had a diuretic effect as reflected by the decrease in urine specific gravity. This effect was more accentuated with one of the diets under study and was likely due to its high salt content (diet2). The increase in urine output resulted in a modest but significant decrease in plasma creatinine concentration in cats receiving diet 2.

The impact of dietary NaCl on water intake has been examined experimentally in cats. Anderson[4] reported that cats fed diets with no (0%) or low (1.4% of dry matter) NaCl had decreased water intake when compared with those fed a high (4.6% dry matter) NaCl diet (130 vs. 250 mL/d). Additionally, diets with elevated sodium content (11.5 to 16.7 g sodium/MJ) have been reported to increase urine volume and decrease both urine specific gravity and urine calcium oxalate relative supersaturation.[48] In humans, high sodium intake has been related to the development of hypertension and osteoporosis.[51,119] However, in healthy cats, feeding diets with high sodium content does not seem to have deleterious consequences in terms of blood pressure and bone mineral

content.[89,120] Thus, increasing salt intake may be a viable option to promote diuresis in healthy cats, however, long term studies are needed to confirm this hypothesis. Moreover, the tolerance to diets with high NaCl content may be reduced in cats with compromised renal function, a common scenario in cats requiring calculolytic diets.[61]

Urine pH was significantly decreased when cats were fed diet 1 but not when they were fed diet 2. A reduction in pH may have influenced the increased urinary excretion of calcium and magnesium in cats fed diet 1, as shown by the correlation study and supported by previous data in the literature.[20] In other species, dietary acidification and metabolic acidosis may increase glomerular filtration of calcium, decrease renal tubular reabsorption of calcium, and promote skeletal mobilization of calcium.[121] In adult cats, dietary acidification has been reported to cause hypercalciuria[20,21] and hypermagnesiuria.[55] The increase in urinary calcium and magnesium seems to be mediated by a decrease in tubular calcium and magnesium reabsorption, independent of PTH, but dependent on changes in net acid excretion. The available data suggest that acid load influences the handling of divalent cations at the thick ascending limb of Henle's loop.[55]

As expected by the lower phosphorus content of the diets, urine phosphorus concentration and FE of phosphorus tended to decrease. It is interesting to note that the decrease was more marked at 30 days. Thus, it seems that the kidney adapts to the lower phosphorus intake and tends to stabilize FE of phosphorus with time. Therefore, the decrease in urine phosphorus concentration, that would help to prevent struvite crystal formation, may be transitory.

An interesting finding of our work was the increase in urine magnesium concentration and FE of magnesium after feeding the calculolytic diets, an effect that was more evident with diet 2. This increase in urinary

magnesium seems both paradoxical, since the diets, especially diet 2, have lower magnesium concentrations and counterproductive to prevent struvite crystal formation. The increase in urine magnesium excretion after feeding diet 1 is likely influenced by the decrease in urine pH, as discussed above. In cats eating diet 2, the increase in magnesium excretion by urine seems to be associated to the high sodium content of the diet and the subsequent increase in urinary sodium excretion. After modification of salt and fluid intake the kidney adapts with corresponding changes in divalent cation excretion; thus, an increase in salt intake has been shown to increase urinary calcium and magnesium loss.[19,64,70] In our study, correlation between sodium and magnesium excretion was very good when the diet was high in sodium but non-existent when sodium content was normal. Thus, our result support the influence of sodium on divalent cation excretion by urine in cats, although in the cats of the present study the effect was mostly seen in magnesium and, to a lower extent, in calcium.

In conclusion, contrary to our hypothesis, the calculolytic diets under study did not seem to negatively affect mineral metabolism. Both diets resulted in an increased urinary excretion of magnesium through different mechanisms: urine acidification and increased sodium load.

CONCLUSIONS

*(From the article: **Pineda C**, Aguilera-Tejero E, Raya AI, Diez E, Rodriguez M, Lopez I. Feline parathyroid hormone: validation of hormonal assays and dynamics of secretion. Domest Anim Endocrinol 2012; 42: 256-264)*

I. Intact PTH and W-PTH assays can be used for measurement of feline PTH. Whole PTH concentration is greater than I-PTH concentration in normal cats. Accuracy, precision, sensibility, reproducibility, and ability to detect changes in PTH in response to changes in calcium are similar in W-PTH and I-PTH assays.

II. The PTH-Ca curve in cats is similar to the canine curve and thus the extensive knowledge on dynamics of PTH secretion in dogs that can be found in the literature could be extrapolated to cats.

*(From the article: **Pineda C**, Aguilera-Tejero E, Guerrero F, Raya AI, Rodriguez M, Lopez I. Mineral metabolism in growing cats: changes in the values of blood parameters with age. J Feline Med Surg 2013; 15: 866-871)*

III. From 3 months of age to adulthood, cats show a decrease in plasma minerals: calcium (both tCa and iCa), phosphorus, and magnesium.

IV. No major changes in PTH are evident in growing kittens, although the W-PTH/I-PTH ratio decreases significantly with age.

V. A reciprocal change in vitamin D metabolites (decrease in calcitriol and increase in calcidiol) has been identified during the growing process. This finding reinforces the need to use adequate age-related reference values for diagnostic purposes, especially when evaluating the vitamin D status in the diagnosis of feline rickets.

*(From the article: **Pineda C**, Aguilera-Tejero E, Raya AI, Guerrero F, Rodriguez M, Lopez I. Assessment of calcitonin response to experimentally induced hypercalcemia in cats. Am J Vet Res 2013; 74: 1514-1521)*

VI. A human calcitonin assay can be used for measurement of feline CT. The assay shows good precision, specificity, and sensitivity, and ability to detect changes in CT response to changes in calcium.

VII. A heterogeneous CT response to hypercalcemia has been observed in the group of cats under study. Two subgroups of cats are distinguished: cats which plasma CT concentration increases during hypercalcemia and cats in which CT concentration remains very low even at very high iCa concentrations (up to 1.60 mM) that are consistent with clinical hypercalcemia in cats.

*(From the study: **Pineda C**, Aguilera-Tejero E, Raya AI, Montes de Oca A, Rodriguez M, Lopez I. Effects of calculolytic diets on feline mineral metabolism)*

VIII. The calculolytic diets under study do not seem to negatively affect mineral metabolism. Although a decrease in plasma calcitriol levels has been found after feeding the diets, the clinical significance of this finding is questionable.

IX. Both calculolytic diets result in an increased urinary excretion of magnesium through different mechanisms: urine acidification and increased sodium load.

ABSTRACT

The present Doctoral Thesis was focused on the study of mineral metabolism in the feline species. Through a series of studies, the relationship between calcium metabolism and the main hormones involved in it has been determined. We also studied the changes in mineral metabolism during the juvenile stage of growing cats and analyzed the effects linked to feeding calculolytic diets on feline mineral metabolism.

The first part of the work was aimed to validate a commercial assay for the quantification of intact (I-PTH) and whole parathyroid hormone (W-PTH) and to characterize the dynamics of PTH secretion, including the assessment of PTH-Ca curve in healthy cats. Thirteen clinically healthy cats were subjected to experimentally induced hypo- and hypercalcemia to study the PTH secretion in response to changes in plasma calcium concentration. The results obtained in these studies showed that the assays used to quantify PTH (I-PTH and W-PTH) were suitable and reliable for the determination of this hormone in feline patients. Furthermore, the feline PTH-Ca curve had a great similarity with canine PTH-Ca curve and thus the previous knowledge on dynamics of PTH secretion in dogs could be extrapolated to cats.

The second part was focused on the study of the major electrolytes and hormones involved in mineral metabolism in growing cats (from 3 to 15 months of age). Based on the hypothesis that growing animals are subjected to changes in mineral metabolism, a total of fourteen healthy cats were studied between 3 and 15 months of age. The concentrations and changes of the main parameters related to mineral metabolism were determined. From 3 months of age to adulthood (15 months) animals experienced significant changes in the concentration of hormones and electrolytes related to mineral metabolism. Therefore, the use of appropriate reference ranges is essential especially when pathological processes are evaluated in young animals.

The third study was focused on the validation of a commercial assay for the determination of calcitonin (CT) in cats and the characterization of the dynamics of CT in response to changes in extracellular calcium through the study of the CT-Ca curve in thirteen healthy cats. A heterogeneous CT response to changes in plasma calcium concentration was observed. More than half of cats did not show an increase in CT concentration in response to hypercalcemia. This striking discovery is quite interesting and future studies exploring CT concentrations in animals with hypercalcemia (idiopathic or otherwise) seem necessary.

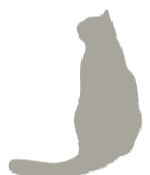
Finally, the fourth study was aimed to determine the influence of two calculolytic diets on mineral metabolism in fourteen healthy cats. It was hypothesized that feeding animals with these diets for long periods may potentially modify mineral metabolism. Contrary to our hypothesis, the calculolytic diets under study did not seem negatively affect the parameters involved in mineral metabolism. Both diets resulted in an increased urinary excretion of magnesium through urine acidification and increased sodium load. Although a decrease in plasma calcitriol levels was evident, the clinical significance of this finding is questionable.

Resumen

La presente Tesis Doctoral se ha centrado en el estudio del metabolismo mineral en la especie felina. A través de una serie de trabajos se ha estudiado en detalle la relación entre el metabolismo del calcio y las principales hormonas involucradas, incluyendo el estudio de los principales cambios que se producen en dichos parámetros durante la etapa juvenil de gatos en crecimiento, para finalmente culminar con el estudio de los posibles efectos ligados a la alimentación con dietas calculolíticas sobre el metabolismo mineral felino.

El primer trabajo se planteó con el objeto de validar un ensayo comercial para la cuantificación de hormona paratiroidea intacta (I-PTH) y completa (W-PTH) y profundizar en el estudio de la dinámica de secreción de PTH y evaluación de la curva PTH-Ca en gatos sanos. Para este estudio se emplearon trece gatos clínicamente sanos los cuales fueron sometidos a hipo- e hipercalcemia inducidas experimentalmente a fin de estudiar la secreción de PTH frente a cambios en la concentración de calcio plasmático. Los resultados obtenidos en esta serie de estudios mostraron que los ensayos empleados para la cuantificación de PTH (I-PTH y W-PTH) fueron adecuados y fiables para la determinación de la concentración de dicha hormona en pacientes felinos. Además, la curva PTH-Ca de los gatos en estudio fue similar a la curva PTH-Ca canina, por lo que los resultados de estudios previos de dinámica de secreción en perros podrían ser extrapolados a la especie felina.

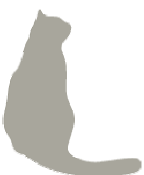
El segundo trabajo se centró en el estudio de las concentraciones de los principales electrolitos y hormonas involucrados en el metabolismo mineral de gatos en crecimiento (desde los 3 a los 15 meses de edad). Basándonos en la hipótesis de que animales en crecimiento están sujetos a cambios del metabolismo fosfocálcico, se estudiaron un total de catorce gatos sanos durante la etapa comprendida entre los 3 y 15 meses de edad a fin de cuantificar los niveles de los principales parámetros relacionados con el metabolismo mineral y determinar qué cambios acontecen durante



esta etapa. Desde los 3 meses de edad hasta la edad adulta (15 meses) los animales experimentaron cambios significativos en las concentraciones de las hormonas y electrolitos relacionados con el metabolismo mineral, por lo que la aplicación de rangos de referencia adecuados resulta imprescindible especialmente cuando se evalúan procesos patológicos en animales jóvenes.

El tercer trabajo se planteó con la finalidad de validar un ensayo comercial para la determinación de calcitonina (CT) en gatos y evaluar la dinámica de secreción de CT en respuesta a cambios en el calcio extracelular mediante el estudio de la curva CT-Ca en trece gatos clínicamente sanos. Se encontró una respuesta heterogénea de CT frente a cambios en la concentración plasmática de calcio. Más de la mitad de los gatos no mostró un incremento en la concentración de CT en respuesta a hipercalcemia. Este hallazgo es realmente interesante y anima a realizar estudios futuros relacionados con la determinación de CT en animales con hipercalcemia idiopática o de otra causa.

Por último, el cuarto trabajo se centró en la influencia de dos dietas calculolíticas sobre el metabolismo mineral de un grupo de catorce gatos sanos ya que se ha planteado la hipótesis de que animales alimentados durante periodos prolongados con dichas dietas pueden desarrollar alteraciones del metabolismo mineral. Contrario a nuestra hipótesis, las dietas calculolíticas bajo estudio no afectaron de manera negativa a los parámetros involucrados en el metabolismo mineral. Ambas dietas provocaron un incremento de la excreción urinaria de magnesio a través de la acidificación urinaria y del incremento de la carga de sodio. Aunque se evidenció un descenso en los niveles de calcitriol, la importancia clínica de este hallazgo es cuestionable.



REFERENCES

1. Aguilera-Tejero E, Sanchez J, Almaden Y, Mayer-Valor R, Rodriguez M, Felsenfeld AJ. Hysteresis of the PTH-calcium curve during hypocalcemia in the dog: effect of the rate and linearity of calcium decrease and sequential episodes of hypocalcemia. *J Bone Miner Res* 1996; 11: 1226-1233.

2. Aguilera-Tejero E, Lopez I, Estepa JC, Mayer-Valor R, Almaden Y, Concepcion MT, Felsenfeld AJ, Rodriguez M. Mineral metabolism in healthy geriatric dogs. *Res Vet Sci* 1998; 64: 191-194.

3. Alon US. Clinical practice. Fibroblast growth factor (FGF)23: a new hormone. *Eur J Pediatr* 2011; 170: 545-554.

4. Anderson RS. Water balance in the dog and cat. *J Small Anim Pract* 1982; 23: 588-598.

5. Barber PJ, Elliott J, Torrance AG. Measurement of feline intact parathyroid hormone: assay validation and sample handling studies. *J Small Anim Pract* 1993; 34: 614-620.

6. Barber PJ, Torrance AG, Elliott J. Carboxyl fragment interference in assay of feline parathyroid hormone. *J Vet Intern Med* 1994; 8: 168.

7. Barber PJ, Elliott J. Feline chronic renal failure: calcium homeostasis in 80 cases diagnosed between 1992 and 1995. *J Small Anim Pract* 1998; 39: 108-116.

8. Barber PJ. Disorders of the parathyroid glands. *J Feline Med Surg* 2004; 6: 259-269.

9. Bas S, Bas A, Estepa JC, Mayer-Valor R, Rodriguez M, Aguilera-Tejero E. Parathyroid gland function in the uremic rabbit. *Domest Anim Endocrinol* 2004; 26: 99-110.

10. Bateman S. Disorders of magnesium: magnesium deficit and excess. In: DiBartola SP ed. *Fluid, electrolyte, and acid-base disorders in small animal practice*, 4th edition. Elsevier. St. Louis, Missouri. 2011; 210-226.

11. Bell O, Silver J, Naveh-Many T. Parathyroid hormone, from gene to protein. In: Naveh-Many T ed. *Molecular Biology of the Parathyroid*, Kluwer Academic/Plenum Publishers. New York. 2005; 8-28.

12. Bolliger AP, Graham PA, Richard V, Rosol TJ, Nachreiner RF, Refsal KR. Detection of parathyroid hormone-related protein in cats with humoral hypercalcemia of malignancy. *Vet Clin Pathol* 2002; 31: 3-8.

13. Bouillon R, Decallonne B. The white adipose tissue connection with calcium and bone homeostasis. *J Bone Miner Res* 2010; 25: 1707-1710.

14. Breslau NA. Normal and abnormal regulation of 1,25-$(OH)_2D$ synthesis. *Am J Med Sci* 1988; 296: 417-425.

15. Brown EM. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *J Clin Endocrinol Metab* 1983; 56: 572-581.

16. Brown EM. Extracellular Ca^{2+} sensing, regulation of parathyroid cell function, and role of Ca^{2+} and other ions as extracellular (first) messengers. *Physiol Rev* 1991; 71: 371-411.

17. Buffington C, Chew D. Effects of diet on cats with non-obstructive lower urinary tract diseases: a review. *J Anim Physiol Anim Nutr (Berl)* 1998; 80: 120-127.

18. Calvo MS, Eastell R, Offord KP, Bergstralh EJ, Burritt MF. Circadian variation in ionized calcium and intact parathyroid hormone: evidence for sex differences in calcium homeostasis. *J Clin Endocrinol Metab* 1991; 72: 69-76.

19. Chan EL, Ho CS, MacDonald D, Ho SC, Chan TY, Swaminathan R. Interrelationships between urinary sodium, calcium, hydroxyproline and serum PTH in healthy subjects. *Acta Endocrinol (Copenh)* 1992; 127: 242-245.

20. Ching SV, Fettman MJ, Hamar DW, Nagode LA, Smith KR. The effect of chronic dietary acidification using ammonium chloride on acid-base and mineral metabolism in the adult cat. *J Nutr* 1989; 119: 902-915.

21. Ching SV, Norrdin RW, Fettman MJ, LeCouteur RA. Trabecular bone remodeling and bone mineral density in the adult cat during chronic dietary acidification with ammonium chloride. *J Bone Miner Res* 1990; 5: 547-556.

22. Civitelli R, Ziambaras K. Calcium and phosphate homeostasis: concerted interplay of new regulators. *J Endocrinol Invest* 2011; 34: 3-7.

23. Cline J. Calcium and vitamin D metabolism, deficiency, and excess. *Top Companion Anim Med* 2012; 27: 159-164.

24. Conlin PR, Fajtova VT, Mortensen RM, LeBoff MS, Brown EM. Hysteresis in the relationship between serum ionized calcium and intact parathyroid hormone during recovery from induced hyper- and

hypocalcemia in normal humans. *J Clin Endocrinol Metab* 1989; 69: 593-599.

25. D'Amour P. Effects of acute and chronic hypercalcemia on parathyroid function and circulating parathyroid hormone molecular forms. *Eur J Endocrinol* 2002; 146: 407-410.

26. D'Amour P, Brossard JH, Rousseau L, Roy L, Gao P, Cantor T. Amino-terminal form of parathyroid hormone (PTH) with immunologic similarities to hPTH(1-84) is overproduced in primary and secondary hyperparathyroidism. *Clin Chem* 2003; 49: 2037-2044.

27. D'Amour P. Circulating PTH molecular forms: what we know and what we don't. *Kidney Int Suppl* 2006; 70: S29-S33.

28. Davey RA, Findlay DM. Calcitonin: physiology or fantasy? *J Bone Miner Res* 2013; 28: 973-979.

29. De Brito Galvao JF, Nagode LA, Schenck PA, Chew DJ. Calcitriol, calcidiol, parathyroid hormone, and fibroblast growth factor-23 interactions in chronic kidney disease. *J Vet Emerg Crit Care (San Antonio)* 2013; 23: 134-162.

30. DiBartola SP, Willard MD. Disorders of phosphorus: hypophosphatemia and hyperphosphatemia. In: DiBartola SP ed. *Fluid, electrolyte, and acid-base disorders in small animal practice*, 4th edition. Elsevier. St. Louis, Missouri. 2011; 195-209.

31. Dittmer KE, Thompson KG. Vitamin D metabolism and rickets in domestic animals: a review. *Vet Pathol* 2011; 48: 389-407.

32. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol* 2005; 289: F8-F28.

33. Estepa JC, Aguilera-Tejero E, Mayer-Valor R, Almaden Y, Felsenfeld AJ, Rodriguez M. Measurement of parathyroid hormone in horses. *Equine Vet J* 1998; 30: 476-481.

34. Estepa JC, Aguilera-Tejero E, Almaden Y, Rodriguez M, Felsenfeld AJ. Effect of rate of calcium reduction and a hypocalcemic clamp on parathyroid hormone secretion: a study in dogs. *Kidney Int* 1999; 55: 1724:1733.

35. Estepa JC, Garfia B, Gao P, Cantor T, Rodriguez M, Aguilera-Tejero E. Validation and clinical utility of a novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone in the horse. *Equine Vet J* 2003; 35: 291-295.

36. Estepa JC, Lopez I, Felsenfeld AJ, Gao P, Cantor T, Rodriguez M, Aguilera-Tejero E. Dynamics of secretion and metabolism of PTH during hypo- and hypercalcaemia in the dog as determined by the “intact” and “whole” PTH assays. *Nephrol Dial Transplant* 2003; 18: 1101-1107.

37. Felsenfeld AJ, Llach F. Parathyroid gland function in chronic renal failure. *Kidney Int* 1993; 43: 771-789.

38. Felsenfeld AJ, Machado L, Rodriguez M. The relationship between serum calcitonin and calcium in the hemodialysis patient. *Am J Kidney Dis* 1993; 21: 292-299.

39. Felsenfeld AJ, Rodriguez M, Aguilera-Tejero E. Dynamics of parathyroid hormone secretion in health and secondary hyperparathyroidism. *Clin J Am Soc Nephrol* 2007; 2: 1283-1305.

40. Fettman MJ, Coble JM, Hamar DW, Norrdin RW, Seim HB, Kealy RD, Rogers QR, McCrea K, Moffat K. Effects of dietary phosphoric acid supplementation on acid-base balance and mineral and bone metabolism in adult cats. *Am J Vet Res* 1992; 53: 2125-2135.

41. Fuleihan GEH, Klerman EB, Brown EN, Choe Y, Brown EM, Czeisler CA. The parathyroid hormone circadian rhythm is truly endogenous – a general clinical research center study. *J Clin Endocrinol Metab* 1997; 82: 281-286.

42. Gao P, Scheibel S, D'Amour P, John MR, Rao SD, Schmidt-Gayk H, Cantor TL. Development of a novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone 1-84: implications for improvement of accurate assessment of parathyroid function. *J Bone Miner Res* 2001; 16: 605-614.

43. Geddes RF, Finch NF, Syme HM, Elliott J. The role of phosphorus in the pathophysiology of chronic kidney disease. *J Vet Emerg Crit Care (San Antonio)* 2013; 23: 122-133.

44. Geisen V, Weber K, Hartmann K. Vitamin D-dependent hereditary rickets type I in a cat. *J Vet Intern Med* 2009; 23: 196-199.

45. Godfrey DR, Anderson RM, Barber PJ, Hewison M. Vitamin D-dependent rickets type II in a cat. *J Small Anim Pract* 2005; 46: 440-444.

46. Gorman ME. Clinical chemistry of the puppy and kitten. In: Peterson ME, Kutzler MA eds. *Small Animal Pediatrics: the first 12 months of life*. Elsevier Saunders. St Louis, Missouri. 2011; 271-274.

47. Guyetant S, Rousselet MC, Durigon M, Chappard D, Franc B, Guerin O, Saint-Andre JP. Sex-related C cell hyperplasia in the normal human

thyroid: a quantitative autopsy study. *J Clin Endocrinol Metab* 1997; 82: 42-47.

48. Hawthorne AJ, Marwell PJ. Dietary sodium promotes increased water intake and urine volume in cats. *J Nutr* 2004; 134: 2128S-2129S.

49. Hazewinkel HAW, Schoenmakers I, Pelling D, Snijdelaar M, Wolfswinkel J, Mol JA. Biological potency and radioimmunoassay of canine calcitonin. *Domest Anim Endocrinol* 1999; 17: 333-344.

50. Hazewinkel HAW, Tryfonidou MA. Vitamin D_3 metabolism in dogs. *Mol Cell Endocrinol* 2002; 197: 23-33.

51. Heaney PR. Role of dietary sodium in osteoporosis. *J Am Coll Nutr* 2006; 25: 271S-276S.

52. Heath H 3rd, Sizemore GW. Plasma calcitonin in normal man. Differences between men and women. *J Clin Invest* 1977; 60: 1135-1140.

53. Henik RA, Forrest LJ, Friedman AL. Rickets caused by excessive renal phosphate loss and apparent abnormal vitamin D metabolism in a cat. *J Am Vet Med Assoc* 1999; 215: 1644-1649.

54. Hollis BW, Kamerud JQ, Kurkowski A, Beaulieu J, Napoli JL. Quantification of circulating 1,25-dihydroxyvitamin D by radioimmunoassay with 125I-labeled tracer. *Clin Chem* 1996; 42: 586-592.

55. Houillier P, Normand M, Froissart M, Blanchard A, Jungers P, Paillard M. Calciuric response to an acute acid load in healthy subjects and hypercalciuric calcium stone formers. *Kidney Int* 1996; 50: 987-997.

56. Houston DM, Rinkardt NE, Hilton J. Evaluation of the efficacy of a commercial diet in the dissolution of feline struvite bladder uroliths. *Vet Ther* 2004; 5: 187-201.

57. Hurwitz S. Homeostatic control of plasma calcium concentration. *Crit Rev Biochem Mol Biol* 1996; 31: 41-100.

58. Institute of Medicine (IOM). *Dietary reference intakes for calcium and vitamin D.* Washington, DC. The National Academies Press (USA). 2011.

59. Inzerillo AM, Zaidi M, Huang CL. Calcitonin: the other thyroid hormone. *Thyroid* 2002; 12: 791-798.

60. Kano K, Yoshida H, Yata J, Suda T. Age and seasonal variations in the serum levels of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D in normal humans. *Endocrinol Jpn* 1980; 27: 215-221.

61. Kerr KR. Companion Animals Symposium: dietary management of feline lower urinary tract symptoms. *J Anim Sci* 2013; 91: 2965-2975.

62. Kirk CA, Jewell DR, Lowry SR. Effects of sodium chloride on selected parameters in cats. *Vet Ther* 2006; 7: 333-346.

63. Kraft W, Hartmann K, Dereser R. [Age dependency of laboratory values in dogs and cats. II. Electrolytes in serum]. *Tierarztl Prax* 1996; 24: 169-173 [article in German].

64. Lee CT, Lien YH, Lai LW, Ng HY, Chiou TT, Chen HC. Variations of dietary salt and fluid modulate calcium and magnesium transport in the renal distal tubule. *Nephron Physiol* 2012; 122: 19-27.

65. Levy JK, Crawford PC, Werner LL. Effect of age on reference intervals of serum biochemical values in kittens. *J Am Vet Med Assoc* 2006; 228: 1033-1037.

66. Lopez I, Aguilera-Tejero E, Estepa JC, Bas S, Mayer-Valor R, Jimenez A, Rodriguez M. Diurnal variations in the plasma concentration of parathyroid hormone in dogs. *Vet Rec* 2005; 17: 344-347.

67. Lulich JP, Osborne CA, Thumchai R, Lekcharoensuk C, Ulrich LK, Koehler LA, Bird KA, Swanson LL, Nakagawa Y. Epidemiology of canine calcium oxalate uroliths. Identifying risk factors. *Vet Clin North Am Small Anim Pract* 1999; 29: 113-122.

68. MacKenzie JM, Crawford J, Ghantous S. Successful therapy of vitamin D-dependant rickets in a kitten. *J Am Anim Hosp Assoc* 2011; 47: 290-293.

69. Major GC, Chaput JP, Ledoux M, St-Pierre S, Anderson GH, Zemel MB, Tremblay A. Recent developments in calcium-related obesity research. *Obes Rev* 2008; 9: 428-445.

70. Massey LK, Whiting SJ. Dietary salt, urinary calcium, and bone loss. *J Bone Miner Res* 1996; 11: 731-736.

71. Mayer GP, Hurst JG. Sigmoidal relationship between parathyroid hormone secretion rate and plasma calcium concentration in calves. *Endocrinology* 1978; 102: 1036-1042.

72. Mellanby RJ, Mee AP, Berry JL, Herrtage ME. Hypercalcaemia in two dogs caused by excessive dietary supplementation of vitamin D. *J Small Anim Pract* 2005; 46: 334-338.

73. Midkiff AM, Chew DJ, Randolph JF, Center SA, DiBartola SP. Idiopathic hypercalcemia in cats. *J Vet Intern Med* 2000; 14: 619-626.

74. Mol JA, Kwant MM, Arnold IC, Hazewinkel HA. Elucidation of the sequence of canine (pro)-calcitonin. A molecular biological and protein chemical approach. *Regul Pept* 1991; 35: 189-195.

75. Morris JG, Earle KE, Anderson PA. Plasma 25-hydroxyvitamin D in growing kittens is related to dietary intake of cholecalciferol. *J Nutr* 1999; 129: 909-912.

76. Mundy GR, Guise TA. Hormonal control of calcium homeostasis. *Clin Chem* 1999; 45: 1347-1352.

77. Naan EC, Kirpensteijn J, Kooistra HS, Peeters ME. Results of thyroidectomy in 101 cats with hyperthyroidism. *Vet Surg* 2006; 35: 287-293.

78. National Research Council. Nutrient requirements of dogs and cats. The National Academies Press. Washington, DC, USA. 2006; 204-205.

79. Nemeth EF. The parathyroid polyhormone hypothesis revisited. *Kidney Int Suppl* 2006; 70: S22-S28.

80. O'Brien MA, Jackson MW. Vitamin D and the immune system: beyond rickets. *Vet J* 2012; 194: 27-33.

81. Osborne CA, Polzin DJ, Kruger JM, Lulich JP, Johnston GR, O'Brien TD. Relationship of nutritional factors to the cause, dissolution, and prevention of feline uroliths and urethral plugs. *Vet Clin North Am Small Anim Pract* 1989; 19: 561-581.

82. Phillips AM, Fawcett AC, Allan GS, Wilkinson M, Fraser DR, Malik R. Vitamin D-dependent non-type 1, non-type 2 rickets in a 3-month-old Cornish Rex kitten. *J Feline Med Surg* 2011; 13: 526-531.

83. Pineda C, Aguilera-Tejero E, Raya AI, Diez E, Rodriguez M, Lopez I. Feline parathyroid hormone: validation of hormonal assays and dynamics of secretion. *Domest Anim Endocrinol* 2012; 42: 256-264.

84. Potts JR, Bringhurst FR, Gardella T, Nussbaum S, Segre G, Kronenberg H. Parathyroid hormone: physiology, chemistry, biosynthesis, secretion, metabolism and mode of action. In: DeGroot LJ ed. *Endocrinology*, 3rd edition. W. B. Saunders. Philadelphia. 1995; 920-966.

85. Poujeol P, Touvay C, Roinel N, de Rouffignac C. Stimulation of renal magnesium reabsorption by calcitonin in the rat. *Am J Physiol* 1980; 239: F524-F532.

86. Pusoonthornthum R Pusoonthornthum P, Krishnamra N. Calcium-phosphorus homeostasis and changes in parathyroid hormone secretion in cats with various stages of spontaneous chronic renal failure. *Comp Clin Pathol* 2010; 19: 287-293.

87. Ramachandran R, Benfield P, Dhillo WS, White S, Chapman R, Meeran K, Donaldson M, Martin NM. Need for revision of diagnostic limits for medullary thyroid carcinoma with a new immunochemiluminometric calcitonin assay. *Clin Chem* 2009; 55: 2225-2226.

88. Reiter AM, Lewis JR, Okuda A. Update on the etiology of tooth resorption in domestic cats. *Vet Clin North Am Small Anim Pract* 2005; 35: 913-942.

89. Reynolds BS, Chetboul V, Nguyen P, Testault I, Concordet DV, Carlos Sampedrano C, Elliott J, Trehiou-Sechi E, Abadie J, Biourge V, Lefebvre HP. Effects of dietary salt intake on renal function: a 2-year study in healthy aged cats. *J Vet Intern Med* 2013; 27: 507-515.

90. Rodriguez M, Felsenfeld AJ, Torres A, Pederson L, Llach F. Calcitonin, an important factor in the calcemic response to parathyroid hormone in the rat. *Kidney Int* 1991; 40: 219-225.

91. Root AW, Harrison HE. Recent advances in calcium metabolism. I. Mechanisms of calcium homeostasis. *J Pediatr* 1976; 88: 1-18.

92. Rosol TJ, Chew DJ, Nagode LA, Capen CC. Pathophysiology of calcium metabolism. *Vet Clin Pathol* 1995; 24: 49-63.

93. Rosol TJ, Capen CC. Pathophysiology of calcium, phosphorus, and magnesium metabolism in animals. *Vet Clin North Am Small Anim Pract* 1996; 26: 1155-1184.

94. Roth SI, Capen CC. Ultraestructural and functional correlations of the parathyroid gland. *Int Rev Exp Pathol* 1974; 13: 161-221.

95. Rourke KM, Kohn CW, Levine AL, Rosol TJ, Toribio RE. Rapid calcitonin response to experimental hypercalcemia in healthy horses. *Domest Anim Endocrinol* 2009; 36: 197-201.

96. Sanchez J, Aguilera-Tejero E, Estepa JC, Almaden Y, Rodriguez M, Felsenfeld AJ. A reduced PTH response to hypocalcemia after a short period of hypercalcemia: a study in dogs. *Kidney Int Suppl* 1996; 57: S18-22.

97. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium. An update on physiological, clinical and analytical aspects. *Clin Chim Acta* 2000; 294: 1-26.

98. Savary KC, Price GS, Vaden SL. Hypercalcemia in cats: a retrospective study of 71 cases (1991-1997). *J Vet Intern Med* 2000; 14: 184-189.

99. Schenck PA. Fractionation of canine serum magnesium. *Vet Clin Pathol* 2005; 34: 137-139.

100. Schenck PA, Chew DJ. Prediction of serum ionized calcium concentration by serum total calcium measurement in cats. *Can J Vet Res* 2010; 74: 209-213.

101. Schenck PA, Chew DJ, Nagode LA, Rosol TJ. Disorders of calcium: hypercalcemia and hypocalcemia. In: DiBartola SP ed. *Fluid, electrolyte, and acid-base disorders in small animal practice*, 4th edition. Elsevier. St. Louis, Missouri. 2011; 122-194.

102. Schoenmakers I, Nap RC, Mol JA, Hazewinkel HA. Calcium metabolism: an overview of its hormonal regulation and interrelation with skeleteal integrity. *Vet Q* 1999; 21: 147-153.

103. Schreiner CA, Nagode LA. Vitamin D-dependent rickets type 2 in a four-month-old cat. *J Am Vet Med Assoc* 2003; 222: 337-339.

104. Schropp DM, Kovacic J. Phosphorus and phosphate metabolism in veterinary patients. *J Vet Emerg Crit Care (San Antonio)* 2007; 17: 127-134.

105. Seino Y, Shimotsuji T, Yamaoka K, Ishida M, Ishii T, Matsuda S, Ikehara C, Yabuuchi H, Dokoh S. Plasma 1,25-dihydroxyvitamin D

concentrations in cords, newborns, infants, and children. *Calcif Tissue Int* 1980; 30: 1-3.

106. Shinki T, Ueno Y, DeLuca HF, Suda T. Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D3-1alpha-hydroxylase gene in normocalcemic rats. *Proc Natl Acad Sci USA* 1999; 6: 8253-8258.

107. Tanner E, Langley-Hobbs SJ. Vitamin D-dependent rickets type 2 with characteristic radiographic changes in a 4-month-old kitten. *J Feline Med Surg* 2005; 7: 307-311.

108. Tomsa K, Glaus T, Hauser B, Flückiger M, Arnold P, Wess G, Reusch C. Nutritional secondary hyperparathyroidism in six cats. *J Small Anim Pract* 1999; 40: 533-539.

109. Toribio RE, Kohn CW, Chew DJ, Capen CC, Rosol TJ. Cloning and sequence analysis of the complementary DNA for feline preproparathyroid hormone. *Am J Vet Res* 2002; 63: 194-197.

110. Toribio RE, Kohn CW, Sams RA, Capen CC, Rosol TJ. Hysteresis and calcium set-point for the calcium parathyroid hormone relationship in healthy horses. *Gen Comp Endocrinol* 2003; 15: 279-288.

111. Toribio RE, Kohn CW, Leone GW, Capen CC, Rosol TJ. Molecular cloning and expression of equine calcitonin, calcitonin gene-related peptide-I, and calcitonin gene-related peptide-II. *Mol Cell Endocrinol* 2003; 199: 119-128.

112. Torrance AG, Nachreiner R. Human-parathormone assay for use in dogs: validation, sample handling studies, and parathyroid function testing. *Am J Vet Res* 1989; 50: 1123-1127.

113. Torres A, Rodriguez M, Felsenfeld A, Martin-Malo A, Llach F. Sigmoidal relationship between calcitonin and calcium: studies in normal, parathyroidectomized, and azotemic rats. *Kidney Int* 1991; 40: 700-704.

114. Vetter T, Lohse MJ. Magnesium and the parathyroid. *Curr Opin Nephrol Hypertens* 2002; 11: 403-410.

115. Wang W, Lewin E, Olgaard K. Rate-dependency of calcitonin secretion in response to increased plasma Ca^{2+}. *Eur J Clin Invest* 2002; 32: 669-673.

116. Wang W, Lewin E, Olgaard K. Role of calcitonin in the rapid minute-to-minute regulation of plasma Ca^{2+} homeostasis in the rat. *Eur J Clin Invest* 2002; 32: 674-681.

117. Wehner A, Katzenberger J, Groth A, Dorsch R, Koelle P, Hartmann K, Weber K. Vitamin D intoxication caused by ingestion of commercial cat food in three kittens. *J Feline Med Surg* 2013; 15: 730-736.

118. Williams TL, Elliott J, Syme HM. Calcium and phosphate homeostasis in hyperthyroid cats: associations with development of azotaemia and survival time. *J Small Anim Pract* 2012; 53: 561-571.

119. Woo J, Kwok T, Leung J, Tang N. Dietary intake, blood pressure and osteoporosis. *J Hum Hypertens* 2009; 23: 451-455.

120. Xu H, Laflamme DP, Long GL. Effects of dietary sodium chloride on health parameters in mature cats. *J Feline Med Surg* 2009; 11: 435-441.

121. Zerwekh JE, Pak CY. Mechanisms of hypercalciuria. *Pathobiol Annu* 1982; 12: 185-201.

122. Zofkova I, Kancheva RL. The relationship between magnesium and calciotropic hormones. *Magnes Res* 1995; 8: 77-84.