Review

A feline-focused review of chronic kidney disease-mineral and bone disorders – Part 2: Pathophysiology of calcium disorder and extraosseous calcification

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Abstract

Derangements in mineral metabolism are one of the main entities in chronic kidney disease-mineral and bone disorder (CKD-MBD). This is the second of a two-part review of the physiology and pathophysiology of calcium homeostasis in feline CKD-MBD. While dysregulation in calcium homeostasis is known to contribute to the development of vascular calcification in CKD, evidence characterising the relationship between serum calcium concentration and nephrocalcinosis and nephrolithiasis is limited. Recently, fibroblast growth factor 23 (FGF23) and α-Klotho have gained increased research interest and been shown to be important biomarkers for the prediction of CKD progression in human patients. However, conflicting evidence exists on their role in calcium homeostasis and vascular and soft tissue calcification. This review details the pathophysiology of calcium disorders associated with CKD-MBD and its implications on vascular and soft tissue mineralisation in human and feline patients. Further prospective studies investigating the clinical consequences of calcium disturbances in cats with CKD are warranted and this may provide additional insight into the pathophysiology of feline CKD-MBD.

Keywords: α-Klotho; CKD-MBD; FGF23; Nephrocalcinosis; Vascular calcification

Introduction

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a phenomenon that has originated from human medicine that characterises the disturbances in mineral and bone metabolism resulting from chronic kidney disease (CKD) (Moe et al., 2006). Calcium homeostasis is tightly regulated by the actions of various hormones, including parathyroid hormone (PTH), calcitriol (a.k.a. 1,25 dihydroxycholecalciferol or 1,25 dihydroxyvitamin D_3), fibroblast growth factor 23 (FGF23), α-Klotho, and calcitonin (Rodriguez et al., 1991; Lu et al., 2008; Khuituan et al., 2012). Metabolism of these hormones is altered in CKD patients due to phosphate retention (Tanaka and Deluca, 1973; Larsson et al., 2003; Centeno et al., 2019), and, in turn, these changes exert influence over calcium homeostasis. Extraosseous calcification is a common complication in human patients with advanced CKD, attributable, at least in part, to derangements in mineral and hormonal metabolism (Yamada et al., 2007). In this review, we aim to summarise the published evidence of calcium and hormonal disturbances associated with CKD in humans and cats and propose areas where additional research is needed to advance our knowledge of feline CKD-MBD.

Mineral and hormonal disturbances in CKD-MBD

Secondary renal hyperparathyroidism in humans

In CKD, phosphate retention occurs secondary to a reduction in glomerular filtration rate (GFR) as the number of functioning nephrons declines (Slatopolsky, 2011). Phosphate retention stimulates secretion of phosphaturic hormones PTH and FGF23 both directly and indirectly via inhibition of renal calcitriol production (Slatopolsky et al., 1996; Liu et al., 2006; Centeno et al., 2019). In the early stages of CKD excess production of FGF23 maintains physiological phosphate concentrations (Gutierrez et al., 2005). As CKD progresses, secondary renal hyperparathyroidism (SRHP) develops (Almaden et al., 1998), with increased serum PTH

concentrations being an indicator of advanced disease (Gutierrez et al., 2005). A large cohort study demonstrated increasing prevalence of hyperparathyroidism with declining GFR (Levin et al., 2007); with increases in PTH beginning to occur before significant calcium and phosphate derangements (i.e. hypocalcaemia and hyperphosphataemia) are observed in human CKD patients. As GFR continues to decline, compensatory mechanisms no longer maintain physiological serum phosphate concentrations, leading to hyperphosphataemia. By the law of mass action, this lowers extracellular ionised calcium (Forman and Lorenzo, 1991) and further stimulates PTH secretion. Over time, down-regulation of parathyroid Vitamin D receptor (VDR) and calcium sensing receptor (CaSR) occurs; driven, at least partly, by calcitriol reduction (Lee et al., 2018; Uchiyama et al., 2020). This further attenuates the response of the parathyroid glands to the inhibitory actions of calcitriol and ionised calcium and contributes to the pathogenesis of SRHP (Korkor, 1987; Brown et al., 1989; Gogusev et al., 1997). Alterations in calcitrophic hormones levels, namely PTH, calcitriol and FGF23 continues to have an effect on calcium homeostasis (Fig. 1). Elevation in PTH results in increased renal phosphate excretion and enhanced renal and intestinal calcium (re)absorption, and bone resorption of calcium, resulting in elevated plasma calcium (see part 1 for further details).

Secondary renal hyperparathyroidism in cats

In one cross-sectional study of 80 cats at various stages of CKD hyperparathyroidism prevalence was documented to be 84%, with all ‘end-stage’ CKD cats (i.e. clinically dehydrated, anorexic, and with survival times of less than 21 days following diagnosis) having evidence of SRHP (Barber and Elliott, 1998). SRHP is also found in cats prior to the development of azotaemia, even in the absence of mineral derangement (Finch et al., 2012). However, a more recent retrospective cross-sectional study of 79 cats with CKD found that a large proportion of cats with International Renal Interest Society (IRIS) Stage 2 CKD had

normal PTH concentrations and cats with IRIS Stage 4 CKD had significantly higher PTH concentrations compared to non-azotaemic age-matched control cats (Geddes et al., 2013).

FGF23-α-Klotho endocrine axis in humans with CKD

Increasing FGF23 is associated with declining renal function (Larsson et al., 2003; Marsell et al., 2008). In early CKD, FGF23 increases prior to the onset of increased phosphate and PTH (Isakova et al., 2011). Although the phosphaturic function of FGF23 initially maintains plasma phosphate balance, it is implicated in the development of SRHP as a consequence of the FGF23-induced reduction in calcitriol, an important negative regulator of PTH synthesis (Shigematsu et al., 2004; Gutierrez et al., 2005; Hasegawa et al., 2010; Van Husen et al., 2010). However, FGF23 also exerts direct and indirect, via local activation of vitamin D, inhibitory effects on PTH synthesis and secretion (Ben-Dov et al., 2007; Krajisnik et al., 2007). The inhibitory effects of FGF23 are attenuated in hyperplastic parathyroid glands, through downregulation of the FGF23 receptor and its co-receptor, α-Klotho (Fig. 1) (Canalejo et al., 2010; Galitzer et al., 2010; Komaba et al., 2010). This phenomenon may underlie SRHP pathogenesis and may explain the concomitant increases in both FGF23 and PTH concentrations in CKD patients.

In contrast to FGF23, soluble α-Klotho protein (s-Klotho) serum concentrations are lower in human CKD patients compared to healthy controls (El Saeeda et al., 2018). Studies support an incremental reduction in s-Klotho with advancing CKD stage (Rotondi et al., 2015; El Saeeda et al., 2018). Reductions occur even in early stage disease (Shimamura et al., 2012) and higher s-Klotho concentrations are associated with lower risk of decline in renal function (Drew et al., 2017), suggesting that s-Klotho may be useful to predict CKD progression from its early stages (Shimamura et al., 2012). An early onset of α-Klotho deficiency may contribute

to the development of FGF23 resistance and a maladaptive increase in FGF23 production (Olauson et al., 2012; Sakan et al., 2014). In support of this, several prospective studies identified a low baseline s-Klotho concentration as an independent risk factor for an adverse kidney disease outcome in CKD patients (Kim et al., 2013; Liu et al., 2018). However, using the same assay methodology as previously described (Yamazaki et al., 2010), Seiler et al. (2013) found no association between declining kidney function and plasma s-Klotho, while Sugiura et al. (2011) identified increasing s-Klotho with decreasing GFR. The complex interplay between FGF23 and α-Klotho in CKD remains to be further elucidated.

FGF23-α-Klotho endocrine axis in cats with CKD

Over the last decade, there has been increased interest in the feline FGF23-α-Klotho axis. Plasma FGF23 concentrations were increased in cats with early-stage non-azotaemic CKD (plasma creatinine <177 μmol/L; with progression to azotaemic CKD within 12 months), suggesting FGF23 may be a useful predictor of development of azotaemia, although there was marked overlap in FGF-23 measurements between groups (Finch et al., 2013). A positive relationship between plasma FGF23 and PTH concentrations was also identified (Finch et al., 2013). In geriatric cats, plasma FGF23 levels increase in parallel with increasing IRIS stage, a surrogate for decreasing renal function (Geddes et al., 2013), comparable to findings in dogs (Harjes et al., 2017; Miyakawa et al., 2020). Furthermore, when comparing cats with the same IRIS stage of azotaemic kidney disease, cats with hyperphosphataemia had significantly higher FGF23 concentrations than those with normophosphataemia (Geddes et al., 2013). Similar to findings in humans, increased FGF23 concentrations are independently associated with higher risks of disease progression and death in CKD cats (Geddes et al., 2015).

Renal expression of α-Klotho by immunocytochemistry in the normal feline kidney was recently reported, with strong expression detected in distal tubules and moderate expression detected in proximal tubules. No α-Klotho expression was detected in the glomeruli (Lawson et al., 2018). As in humans, gene expression of α-Klotho in renal tissue was significantly decreased in cats with more advanced CKD (van den Broek, 2018). Nevertheless, no association between plasma s-Klotho concentrations and renal function was found in cats (Sargent et al., 2020).

Vitamin D deficiency in humans with CKD

Decreased serum vitamin D concentrations (i.e. calcidiol and calcitriol) are commonly observed among CKD patients, with an increased prevalence in end-stage renal disease (ESRD) (Del Valle et al., 2007; Wolf et al., 2007; Kim et al., 2014), although it may be documented as early as Stage 2. (Levin et al., 2007). Progressive reduction in calcitriol is attributable, initially, to the inhibitory effects of increasing FGF23 and hyperphosphataemia and, latterly, to declining functional renal mass (Gutierrez et al., 2005). FGF23 exerts dual effects upon calcitriol production; in a Klotho-dependent manner, it downregulates 1α-hydroxylase whilst stimulating renal expression of 24-hydroxylase (see part 1 for further details). Therefore, as GFR progressively declines, calcitriol concentrations decrease thereby contributing to SRHP. This occurs directly because of diminished inhibitory genomic actions of calcitriol on PTH transcription and synthesis, as well as indirectly via the decrease in circulating ionised calcium secondary to a reduction in calcitriol-mediated intestinal calcium absorption. CKD is also characterised by calcitriol-resistance due to parathyroid gland VDR loss, further contributing to SRHP progression (Fig. 1) (Fukuda et al., 1993).

Low serum vitamin D concentrations often associate with high bone turnover and decreased bone mineral density in human patients with CKD (Tomida et al., 2009; Lee et al., 2014). Patients with low serum calcidiol concentrations also have markedly increased serum PTH levels, suggesting a contribution of hyperparathyroidism in the alterations of bone density and development of renal osteodystrophy (Lee et al., 2014). Vitamin D is essential in the maintenance of bone health, in part through PTH suppression.

Vitamin D deficiency in cats with CKD

When uraemic cats and those with 'end-stage' CKD were compared to non-azotaemic control cats serum calcitriol concentrations were significantly lower (Barber and Elliott, 1998); with 80% of 'end-stage' CKD cats demonstrating calcitriol deficiency (calcitriol <9 pg/mL). Ionised hypocalcaemia (<1.18 mmol/L) was also identified in a significant proportion (56%) of these cats with end-stage disease, and all had concurrent hyperparathyroidism (PTH >25.5 pg/mL) (Barber and Elliott, 1998). These findings suggested diminished synthesis of calcitriol in cats with advanced CKD, despite the stimulatory actions from high PTH levels, resulting in ionised hypocalcaemia; although this may also be partly attributable to the concurrent severe hyperphosphataemia.

Hypercalcaemia in cats with CKD

Hypercalcaemia is a co-morbidity observed in cats with CKD. A recent study showed that CKD cats had higher risk of developing total hypercalcaemia (van den Broek et al., 2017), with increasing prevalence observed in cats with advancing azotaemia (Barber and Elliott, 1998). In general, most hypercalcaemic cats have no identifiable underlying aetiology for increased calcium concentrations (Midkiff et al., 2000). It is unclear what role, if any, the presence of hypercalcaemia has in the development or progression of CKD in cats.

Dietary phosphate restriction is recognised as a fundamental component of the management of CKD in humans (Lou et al., 2012). Dietary phosphate restriction is also recommended in the management of CKD in cats (Elliott et al., 2000; Plantinga et al., 2005; Ross et al., 2006). However, the disparity between the nutrient requirements for calcium and phosphate as recommended for adult cats by the National Research Council (revised in 2006 and adopted by the European Pet Food Industry Federation in 2018; 0.72 g and 0.64 g per 1000 kcal ME, respectively) and those recommended by the Association of American Feed Control Officials (AAFCO; 1.5g and 1.25 g per 1000 kcal ME, respectively; revised in 2014), is such that some clinical kidney-support diets previously considered to be absolutely 'phosphate-restricted' would now not be. For example, in one study the 'phosphate-restricted' kidney-support diets were reported to have phosphate levels from 0.725 g (dry) to 1.025 g (canned) per 1000 kcal ME and calcium levels from 1.375 g (dry) to 1.7 g (canned) per 1000 kcal ME (Barber et al., 1999). To further complicate comparisons, not all forms of dietary phosphorus are equal, with differing apparent digestibility influenced by the forms of phosphate included and other components of the diet (Coltherd et al., 2019). Thus, although phosphate levels in the kidney-support diets are lower than standard adult maintenance diets, the beneficial effects of each particular formulation when used in the field should be determined by demonstrating their ability to reduce the markers of mineral bone disturbance (Elliott et al., 2000; Ross et al., 2006). Feeding of clinical renal diets may also contribute to hypercalcaemia in some azotaemic cats. This phenomenon was first documented in a prospective study in which two of 15 CKD cats developed total and ionised hypercalcaemia following feeding of a clinical renal diet (described above); subsequent feeding of the cat's regular diet resolved the total hypercalcaemia, although the effect on ionised calcium was not reported (Barber et al., 1999). In another study of 10 cats that developed ionised hypercalcaemia whilst being fed a phosphate-restricted clinical renal

diet (0.8 g per 1000 kcal ME), attenuation of the degree of dietary phosphate restriction (i.e. feeding of a moderately protein- and phosphate-restricted senior diet with 1.5 g per 1000 kcal ME) restored ionised normocalcaemia after a median of 2.2 months in eight, whilst the remaining two had improving calcium concentrations and only short-term follow-up (Geddes et al., 2021). Interestingly, a higher proportion of healthy senior cats (≥9 years) developed ionised hypercalcaemia after initiation of a moderately protein- and phosphate-restricted diet with 1.6 g per 1000 kcal ME (5/26 cats) compared to those eating a control diet (1/28 cats) with >60% higher dietary phosphate content (2.6 g per 1000 kcal ME) (Geddes et al., 2016). However, development of ionised hypercalcaemia was not reported in similar dietary trials among healthy young adult cats (≤8 years), with minimal changes in total or ionised calcium concentrations observed over time (Kienzle et al., 1998; Alexander et al., 2019). The discrepancy between these studies remains uncertain. When comparing a group of CKD cats that had increased total plasma calcium concentrations during the first 200 days following transition to a phosphate-restricted diet (0.7–1.1 g per 1000 kcal ME) to those that maintained their plasma calcium concentrations, only the group with increasing total plasma calcium also had increases in ionised calcium, FGF23, phosphate, creatinine and SDMA, suggesting an association between increasing plasma calcium concentration and progression of kidney disease (Tang et al., 2021); however, the clinical significance of these changes was not evaluated and evaluation over a longer timeframe is necessary.

Extraosseous calcification

Vascular and soft tissue calcification

One of the mechanisms by which hypercalcaemia and hyperphosphataemia may be detrimental to CKD patients is through development of vascular and renal mineralisation (Fig. 2) – a hypothesis which remains to be proven.

Vascular calcification (VC) is a common consequence of CKD in humans characterised by deposition of calcium-phosphate salts in the vessel wall (tunica intima and tunica media), with medial calcification being more specific to CKD-MBD (London et al., 2003; Fox et al., 2006; Shroff et al., 2008). It is a pathological process facilitated by the osteochondrogenic differentiation of vascular smooth muscle cells (VSMCs), VSMC apoptosis and elastin degradation (Proudfoot et al., 2000; Shroff et al., 2008, 2010). VC is highly prevalence in ESRD patients (Raggi et al., 2002; Kraus et al., 2015). Medial wall calcification increases vessel stiffness, resulting in elevated pulse pressure and left ventricular hypertrophy, associated with increased risk of cardiovascular events and mortality (Klassen et al., 2002; London et al., 2003; Go et al., 2004; Paoletti et al., 2016).

Ectopic calcification has traditionally been explained by the precipitation of calcium and phosphate salts from supersaturated fluid when the calcium and phosphate product (Ca x P) exceeds the solubility product. Increased calcium and phosphate concentrations are associated with hydroxyapatite deposition (Fig. 2) (Villa-Bellosta et al., 2011). However, it has become clear that soft tissue calcification is a multifaceted and complex process, regulated by various inducers and inhibitors (Lomashvili et al., 2004, 2006; Babler et al., 2020). Several factors have been implicated in the inhibition of soft tissue mineral deposition, such as Fetuin-A, magnesium, osteoprotegerin, matrix Gla protein, pyrophosphate, and bone morphogenic protein 7. Reductions in these physiological calcification inhibitors are believed to provide a pro-calcific environment and accelerate mineralisation (Lomashvili et al., 2004; Shroff et al., 2008).

Roles of calcium in VC in humans

As detailed above, mineral metabolism disturbances occur even at early stages of CKD and are usually characterised by increased FGF23, α-Klotho, and PTH, and calcitriol deficiency. Increased serum calcium concentrations and the development and progression of VC are closely associated in the CKD population (West et al., 2010). Synergism between calcium and phosphate was found in mediating VSMC calcification, by promoting osteochrondrogenic differentiation of VSMCs, formation of apoptotic bodies, and release and deposition of matrix vesicles (Reynolds et al., 2004; Shroff et al., 2010). In vitro, extracellular calcium concentrations were positively correlated with the mineralisation of human VSMCs under controlled phosphate conditions (Reynolds et al., 2004; Yang et al., 2004), with increased extracellular calcium capable of inducing VSMC calcification independent of, as well as synergistically with, phosphate (Reynolds et al., 2004). Clinically, progressive arterial calcification has been associated with CKD in haemodialysis patients receiving calcium-based phosphate binders (Chertow et al., 2004); whereas this pathology was less prevalent, together with a decreased risk of all-cause mortality, in those treated with non-calcium-based phosphate binders (Chertow et al., 2002; Asmus et al., 2005; Jamal et al., 2013). It is apparent that dysregulation in calcium homeostasis promotes VC in CKD, most likely in synergy with phosphate.

Roles of FGF23 and α-Klotho in VC in humans and other species

FGF23 and α-Klotho, in addition to their homeostatic roles maintaining calcium and phosphate balance, are emerging factors in VC within CKD-MBD (Alexander et al., 2009; Lim et al., 2012; Jimbo et al., 2014). Despite the well-recognised correlation between elevated FGF23 levels and renal dysfunction, FGF23's roles in VC of CKD remains controversial. Several studies identified no association between serum FGF23 and the prevalence or the severity of coronary arterial calcification (CAC) in CKD patients of varying stages (Gutiérrez

et al., 2009; Scialla et al., 2013). However, not all studies agree. Human patient cohort studies (with and without CKD) have found serum FGF23 levels to be independently associated with VC (Nasrallah et al., 2010; Desjardins et al., 2012; Donate-Correa et al., 2019). Haemodialysis patients with increased serum FGF23 concentrations were at greater risk to develop progressive CAC within a one year period (Ozkok et al., 2013). This is further supported by experimental studies indicating that FGF23 potentiates phosphate-induced VC in Klotho-overexpressing VSMCs and rat aortae (Jimbo et al., 2014). In contrast, a direct protective effect of FGF23 on calcifying mouse VSMC has also been demonstrated (Zhu et al., 2013). This is consistent with the findings that FGF23, in an α-Klotho-dependent manner, inhibited human aortic VSMC extracellular matrix calcification (Lim et al., 2012). Based on conflicting evidence from the literature, further investigation is required to fully understand under what circumstances FGF23 exerts a protective or detrimental role in VC.

There is substantial evidence from various species showing the inhibitory effects of α-Klotho on VC, although conflicting results also exist. VC appeared to be a direct effect of α-Klotho deficiency in a mouse CKD model, whilst s-Klotho was found to suppress sodium-dependent phosphate transport and directly inhibit phosphate-induced calcification in rat VSMCs (Hu et al., 2011). α-Klotho also maintained the VSMC phenotype by abrogating the changes in osteochrondrogenic differentiation transcription factors (i.e. decreases in *Pit1*, *Pit2*, and *Runx2* mRNA and an increase in *SM22* mRNA) (Hu et al., 2011). Similarly, α-Klotho suppressed phosphate-induced calcium deposition in bovine aortic VSMCs, and *ex vivo* data from murine aortic rings suggested that inhibition of mammalian target of rapamycin (MTOR)-signalling ameliorated VC through α-Klotho upregulation (Zhao et al., 2015). Reduction of hyperphosphataemia-associated VC in response to stable delivery of circulating s-Klotho was also demonstrated in α-Klotho-null mice (Hum et al., 2017). Additionally, mRNA and protein

expression of α-Klotho were increased in cultured VSMCs under calcifying conditions (Zhu et al., 2013) and increased expression of α-Klotho protein was observed in calcified aortae from a mouse model of VC ($Enpp1^{-/-}$; resulting in reduced levels of the mineralisation inhibitor pyrophosphate) (Zhu et al., 2013). Despite the increasing research interest, it has not been confirmed whether α-Klotho is expressed endogenously in the vasculature; an elegant review by Mencke and Hillebrands on behalf of the NIGRAM consortium summarised the conflicting evidence of α-Klotho expression in the vasculature (Mencke et al., 2017).

The discordant findings regarding both FGF23 and α-Klotho on VC may be attributed to the differences in species, methodologies and detection techniques among studies, including the types of cultured cells and the application of different antibodies. Better understanding of the roles of FGF23 and α-Klotho in the pathophysiology of VC may reveal their potential as novel therapeutic targets in CKD-MBD.

Nephrocalcinosis and nephrolithiasis associated with CKD in humans

Although the relationship between VC and CKD-MBD is well documented in human patients this is not the case for nephrocalcinosis and CKD-MBD. Nephrocalcinosis is characterised by deposition of calcium-phosphate or calcium-oxalate within tubulointerstitial regions, with medullary nephrocalcinosis being the pattern seen in 98% of human cases (Wrong, 2006). Calcium nephrolithiasis refers to the aggregation of calcium crystals in the kidney into visible stones. The pathophysiology of nephrocalcinosis and nephrolithiasis are intimately related; both processes begin from Randall's plaque formation in the renal papillae which act as a nadir for progressive calcification (Randall, 1937; Evan et al., 2003). However, nephrocalcinosis is not a prerequisite for the development of nephrolithiasis, and

nephrolithiasis is not an inevitable consequence of nephrocalcinosis, suggesting distinct pathomechanisms (Wrong, 2006).

Renal calcification is prevalent in CKD patients. Evidence of renal calcium deposition was identified histologically in 72% of non-dialysis and 93% of dialysis patients (Kuzela et al., 1977). This is consistent with a recent study which showed progressively increasing prevalence of nephrocalcinosis in patients with advancing CKD, with over 50% of CKD stage 5 patients and >70% of dialysis patients having evidence (Evenepoel et al., 2015). Interestingly, there are conflicting data on calcium and phosphate concentrations in patients with and without nephrocalcinosis. Some studies suggest no significant difference in serum calcium and phosphate levels between the adult and paediatric patients with and without visceral calcification (Kuzela et al., 1977; Milliner et al., 1991), which has been further supported by a histological study showing that serum calcium was not associated with renal calcium content and renal tubular calcium deposition (Gimenez et al., 1987). In another study, hypercalciuria was a risk factor for nephrocalcinosis (Rönnefarth and Misselwitz, 2000). This suggests a potential predisposing role of calcium disturbances in abnormal renal tubular mineral deposition (Evenepoel et al., 2015). Furthermore, microscopic nephrocalcinosis development appears to begin early in CKD and correlates with the degree of renal impairment (Gimenez et al., 1987; Evenepoel et al., 2015), suggesting a deleterious effect of nephrocalcinosis on kidney function and potential role in accelerating CKD progression.

Similar to nephrocalcinosis, a recent meta-analysis showed an association between nephrolithiasis and CKD progression (Zhe and Hang, 2017). Patients with nephrolithiasis had over two times the risk (relative risk = 2.16) of developing ESRD compared to those without nephrolithiasis (Zhe and Hang, 2017). Higher urine calcium excretion is the most frequent

pathophysiological factor associated with calcium nephrolithiasis (Saponaro et al., 2020). In a study of 176 patients with primary hyperparathyroidism, patients with nephrolithiasis had increased urinary calcium excretion when compared to individuals without evidence of nephrolithiasis; however, no differences in serum total and ionised calcium were detected (Saponaro et al., 2020).

Both nephrocalcinosis and nephrolithiasis are frequent pathological entities affecting the CKD population. However, evidence of an association between extracellular calcium concentration and renal mineralisation in human patients remains equivocal. Further studies are warranted to investigate whether increased serum calcium exerts any deleterious effects on renal function as a consequence of nephrocalcinosis or nephrolithiasis.

Vascular and soft tissue calcification in cats with CKD

Although vascular and soft tissue calcification are widely identified in human CKD patients, predominantly in those with derangements in mineral metabolism, very few studies have been undertaken to evaluate this phenomenon in feline CKD. Cases of concurrent cutaneous calcinosis and renal disease have been described (Anderson et al., 1988; Böhmer et al., 1991). At post-mortem examination, both cats had pathological changes associated with CKD, including medullary nephrocalcinosis documented in one. Interdigital and footpad calcification associated with moderate and severe CKD has also been described in two individual case reports (Jackson and Barber, 1998; Declercq and Bhatti, 2005), and a case series of five cats (Bertazzolo et al., 2003). Although serum PTH was not measured in all cases, the calcium phosphate product was greater than 5.65 $mmol^2/L^2$ (70 mg^2/dL^2) in all seven cats; this is suggestive of metastatic calcification. Radiographic evidence of thoracic and abdominal aorta and gastric wall mineralisation was also present in both cats and one cat, respectively,

where relevant imaging was performed (Bertazzolo et al., 2003). In a larger study, gastric mineralisation within the mucosal lamina propria was found in 14 of 37 (38%) CKD cats at post-mortem examination, with evidence of vascular mineralisation in three (Mcleland et al., 2014). This gastric mineralisation was only present in cats with moderate or severe CKD. Another review of samples collected at post-mortem examination found that cats with IRIS stage 2–4 CKD were ~2.5-times more likely to have nephrocalcinosis as compared to non-azotaemic geriatric control cats (prevalence of 50–58% cf. 21%) (Chakrabarti et al., 2013). However, no association between tubular mineralisation and plasma phosphate concentration was reported and plasma calcium, PTH, vitamin D metabolite, FGF-23 and α-Klotho concentrations were not given. Thus, the prevalence, pathophysiology and implications of soft tissue calcification, including nephrocalcinosis, associated with mineral metabolism disturbances in feline CKD has not been thoroughly investigated. Further studies focusing on this topic may provide an insight into the pathophysiology of feline CKD-MBD.

Recently, a positive association between urolithiasis and feline CKD was identified, with a higher CKD prevalence detected among cats with urolithiasis (Cléroux et al., 2017). Serum SDMA was also shown to be increased in cats with radiological evidence of urolithiasis, as compared to healthy control cats (Hall et al., 2017). However, it remains unknown whether CKD predisposes the formation of uroliths or vice versa. Radiographic evidence of nephroliths was documented in 29% (13/45) of cats with IRIS stage 2–3 CKD (Ross et al., 2006), although in a subsequent study presence of nephroliths was not associated with disease progression and mortality (Ross et al., 2007). However, only cats with stable renal function were included in the latter study and sample size was small. Prospective studies are required to further evaluate the relationship between CKD and nephrolithiasis in cats.

Emerging factors in CKD-MBD

CKD-MBD is a constantly evolving field in both human and feline medicine. In recent years, new factors and pathways associated with CKD-MBD are being explored, including the Wnt-β-catenin signalling pathway, which may link to the pathophysiological consequences of mineral dysregulation, renal osteodystrophy and VC (Holmen et al., 2005; Surendran et al., 2005; Claes et al., 2013; Ryan et al., 2013; Fang et al., 2014; Yang et al., 2015; Carrillo-López et al., 2016). However, controversies and uncertainties exist in current literature and more studies are required to better understand the roles and significance of these emerging factors in CKD-MBD.

Conclusions

Mineral metabolism disturbances are almost ubiquitously present in CKD patients and the severity of these debilitating complications is commonly associated with disease progression and death. During early stage CKD, circulating phosphate and calcium are often maintained within physiological limits by the adaptive responses and interactions of the major regulatory factors involved in CKD-MBD, namely PTH, calcitriol, FGF23 and α-Klotho. However, as renal function continues to decline, these responses become maladaptive and result in mineral derangements. Cats with CKD are at increased risk of developing total hypercalcaemia compared to non-azotaemic cats, but the underlying causes of hypercalcaemia remain to be determined. Increasing evidence has suggested the involvement of dietary phosphate restriction in the development of hypercalcaemia in some azotaemic cats; however, evidence is limited due to the lack of prospective controlled clinical trial data. In human medicine, hypercalcaemia is a well-established risk factor for vascular and soft tissue calcifications in CKD patients. These complications are also associated with declining renal function and increased mortality. However, the prevalence and implications of these

complications have not been thoroughly investigated in feline CKD. Advances in the knowledge of the pathophysiology and implications of calcium derangements associated with feline CKD may allow refinement of management strategies in feline CKD-MBD.

In addition, progressive CKD, even at early stages, is characterised by a rise in serum FGF23 followed by a decline in α-Klotho in human patients. Although a wealth of knowledge has emanated from research into the association of FGF23 and α-Klotho with VC in human CKD patients over the last decades, this relationship has not been explored in feline CKD. Further studies are required to establish the interrelated roles of FGF23 and α-Klotho on calcium handling, as well as vascular and soft tissue calcification, in feline CKD.

Conflict of interest statement

P.K. Tang received PhD studentship funded by Royal Canin SAS. R. Jepson received funding from PetPlan, Feline Foundation for Renal Research, RVC Internal Grant, PetSavers, and consultancy agreements: Boehringer Ingelheim, Merial, CEVA. Speaking honoraria: Boehringer Ingelheim, Hills Pet Nutrition, CEVA. R. Geddes received funding from Petplan, an RVC Internal Grant, The Academy of Medical Sciences and The Winn Feline Foundation; has a consultancy agreement with Boehringer Ingelheim; speaking honoraria from Boehringer Ingelheim. J. Elliott received funding from Consultancies: Elanco Ltd, CEVA Animal Health Ltd, Boehringer Ingelheim Ltd, MSD Animal Health Ltd., Orion Incorp, Idexx Ltd, Nextvet Ltd, Waltham Centre for Pet Nutrition, Kindred Biosciences Inc, Invetx Inc; grant funding from Elanco Ltd, Waltham Centre for Pet Nutrition, Royal Canin SAS, Idexx Ltd., Zoetis Ltd, CEVA Animal Health, Member of the International Renal Interest Society.

Acknowledgement

The authors acknowledge support from Royal Canin SAS for its research contributions on feline chronic kidney disease-mineral and bone disorder. All figures in this review are created with Biorender.com.

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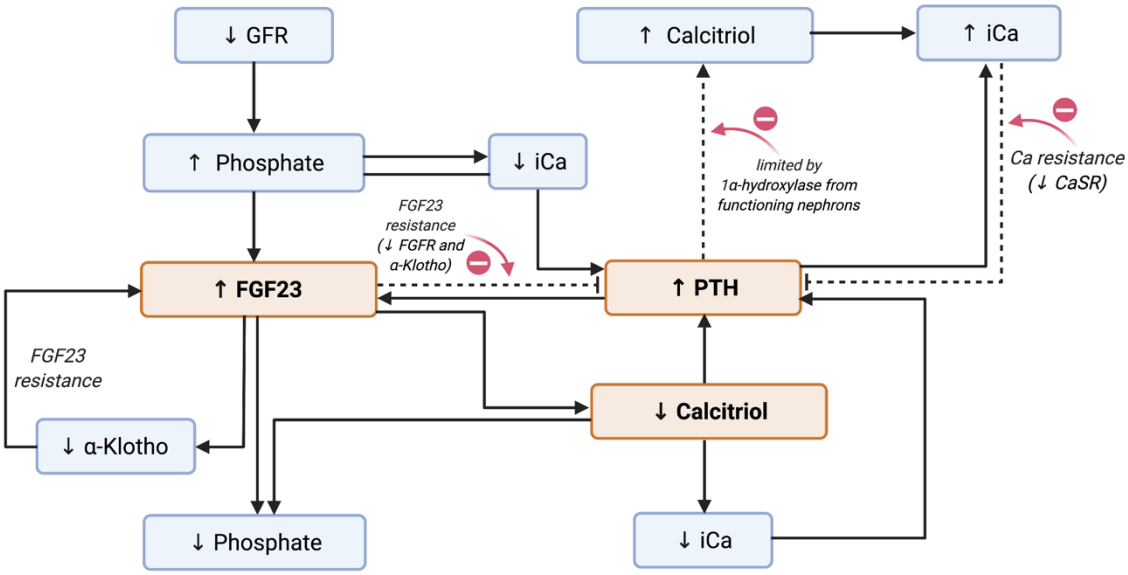
Figure Legends

Fig. 1. Schematic diagram showing the roles of fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH) and calcitriol in calcium homeostasis in chronic kidney disease (CKD). Reduction in glomerular filtration rate (GFR) leads to phosphate retention. This stimulates the production of FGF23, which enhances urinary excretion of phosphate and inhibits the synthesis of calcitriol in the kidney, to maintain plasma phosphate concentration within physiological limits. As GFR continues to decline, accumulation of plasma phosphate, together with increased FGF23, reduced plasma ionised calcium (iCa) and reduced calcitriol, stimulates PTH production and contributes to the development of secondary renal hyperparathyroidism. This is further exacerbated by the diminished inhibitory effects of FGF23 and iCa on PTH secretion due to the reduction in α-Klotho, fibroblast growth factor receptor (FGFR) and calcium-sensing receptor (CaSR) in the parathyroid gland. Despite the continuous stimulatory effect of PTH in advancing CKD, reduction in calcitriol occurs as a consequence of the downregulation of 1α-hydroxylase due the decline in functional renal mass and the inhibitory effects exerted by FGF23, resulting in ionised hypocalcaemia.

Fig. 2. Schematic illustration of the cell-mediated process of vascular calcification (VC). VC is mediated by the deposition of hydroxyapatite, composed of calcium and phosphate, in the vasculature. Calcium and phosphate work synergistically to promote osteochrondrogenic transdifferentiation of vascular smooth muscle cells (VCMCs) and formation of apoptotic bodies to produce a local pro-calcifying environment for VC. Fetuin-A acts as a potent inhibitor of VC by reversibly aggregating with calcium-phosphate precipitates to prevent supersaturation and formation of hydroxyapatite in the blood. Fetuin-A is often downregulated in human CKD population.

1053 Figure 1



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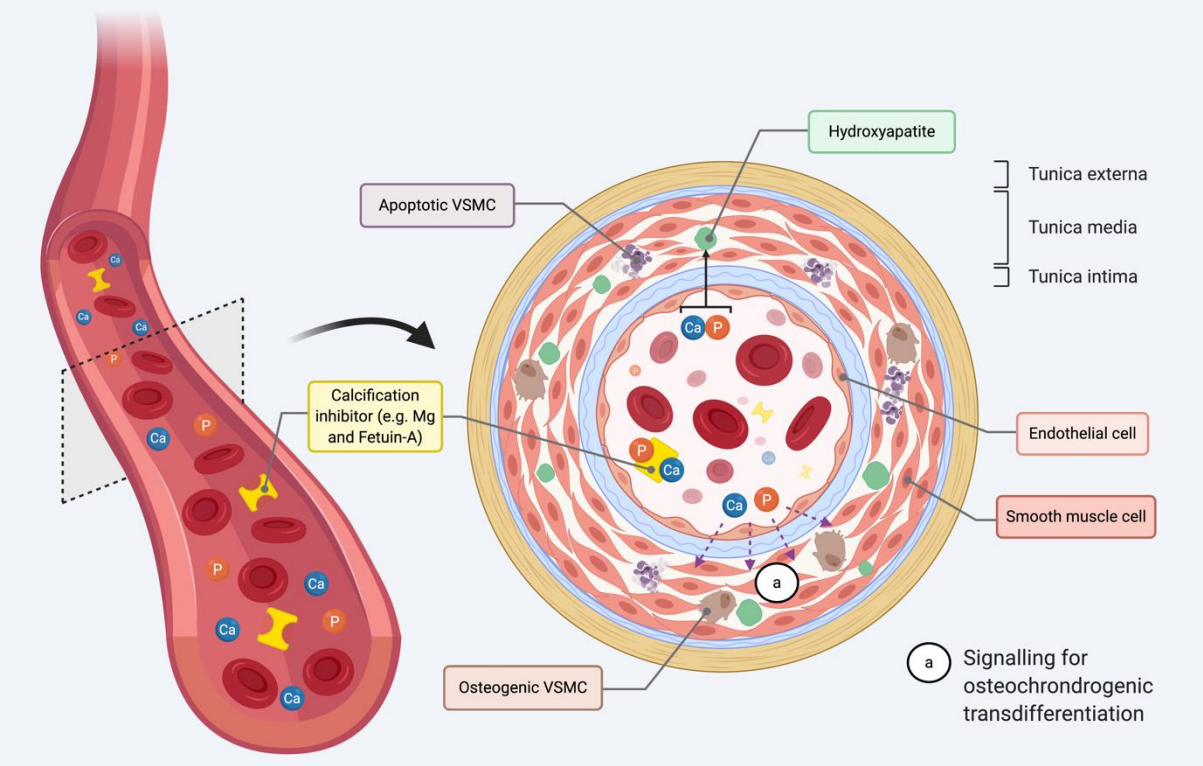
1055 Figure 2



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