FGF23 & Klotho

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CONTENT

KEY POINTS

Fibroblast Growth Factor 23 (FGF23) is a protein synthesized by osteocytes that has a key role in the 'bone-parathyroid-kidney' axis and the regulation of phosphate/calcium metabolism. FGF23 has three main effects: hypophosphatemia (through an inhibition of phosphate reabsorption in the proximal tubule), decreased PTH levels and decreased 1,25 $(OH)_2$ Vitamine D levels (through an inhibition of 1α hydroxylase and an activation of 24 hydroxylase activity in the kidney). Off-targets of FGF23 have also been demonstrated, notably on cardiomyocytes and monocytes. The links between iron metabolism and FGF23 on one hand, and between FGF23 and bone mineralization on the other hand, seem also of importance, although still under investigation.

The single-pass transmembrane Klotho protein, an anti-aging protein, is required *in vivo* for FGF23-mediated receptor activation, at least for its renal effects. However, Klotho also has mineral effects by it-self: it can function as an enzyme modifying the sugar chains of TRPV5 in the distal tubule, leading to increased calcium reabsorption, and it can also directly regulate PTH synthesis. Through alternative splicing or ectodomain shedding of the transmembrane protein, the Klotho protein exists both as a secreted (α-Klotho) and a membrane protein whose extracellular domain could be shed from the cell surface and released into the circulation to act as an endocrine factor, explaining that apart from its key role in phosphate/calcium metabolism regulation, Klotho has also many other roles in general metabolism.

In human diseases, FGF23 as well as Klotho can be deregulated, either in genetic diseases, either in acquired diseases. Four groups can be distinguished: diseases with primary increase of FGF23 levels (e.g., hypophosphatemic rickets or tumor-induced osteomalacia), diseases with primary decrease of FGF23 levels (e.g., hyperphosphatemic tumoral calcinosis), diseases with secondary increase of FGF23 levels (e.g., chronic kidney disease, CKD), and diseases with secondary decrease of FGF23 levels (e.g., VDR deficiency leading to vitamin D-resistant rickets).

A lot of studies have focused on FGF23 and Klotho during CKD, these two biomarkers being indeed the first biomarkers to be deregulated in early CKD (increased FGF23 levels, and decreased Klotho levels). In such a disease, increased FGF23 levels have been shown to be a risk factor for cardiovascular mortality, general mortality, progression of renal disease, resistance to vitamin D analogs, and in transplant patients as a risk factor for acute rejection and graft loss.

Theorically, the use of antiFGF23 agents in CKD patients could be of interest, but results from animal models have been disappointing, enabling the correction of hyperparathyroidism while increasing mortality. In contrast, in a next future, antiFGF23 antibodies may be of interest for genetic diseases such as hypophosphatemic rickets; therapeutic trials in this field are ongoing.

Both FGF23 and Klotho levels can be assessed in blood with immunometric assays: with the second-generation assay and its better accuracy for C-terminal FGF23, the technique is robust but unfortunately it is not available everywhere. For Klotho assessment, the assay measures the portion of α-Klotho that circulates in the systemic circulation after its release from the cell membrane. The clinical relevance of such a circulating α-Klotho remains to be fully understood, since the main biological effects of Klotho are probably mediated by the trans-membrane form of the protein.

Introduction

Fibroblast Growth Factor 23 (FGF23) is a protein synthesized mainly by osteocytes that has been described to have a key role in the 'bone-kidney' axis and the regulation of phosphate/calcium metabolism (1-3). FGF23 acts mainly as a phosphaturic factor and a suppressor of 1α hydroxylase activity in the kidney: it inhibits the expression of type IIa and IIc sodium-phosphate cotransporters on the apical membrane of proximal tubular cells, leading to an inhibition of phosphate reabsorption (4). Moreover, it also inhibits 1α hydroxylase activity whereas it stimulates the 24 hydroxylase activity, thus leading to decreased 1,25 dihydroxyvitamin D serum levels (5). Consequently, these two pathways account together for the hypophosphatemic effect of FGF23. FGF23 was also described as an inhibiting factor of parathyroid hormone (PTH) synthesis (6). Moreover, it is interesting to note that, in contrast to its renal effects, FGF23 can stimulate the local expression of 1α hydroxylase in the parathyroid, suggesting that it can also indirectly down regulate PTH synthesis through an increased local production of calcitriol (7). The single-pass transmembrane Klotho protein, an anti-aging protein, seems required *in vivo* for FGF23-mediated receptor activation, at least for its renal effects (8, 9).

1 FGF23: structure and biochemical properties

FGF23 is a 251 amino-acid protein (molecular weight = 30 kDa) with a 24 amino-acid signal peptide in the N-terminal portion; its chromosomic location is 12p13 in humans. It belongs to the FGF family, in the sub-group of the 'endocrine FGFs' with FGF19 and FGF21 (10): indeed, it shares with all the FGFs a highly conserved sequence but it also has a unique C-terminal structure as well as a specific three-dimensional configuration (i.e., disulfide bound and β sheet), both accounting for its systemic action (10, 11). When it was initially described, FGF23 was thought to be cleaved by a specific metalloproteinase called PHEX (phosphate-regulating gene with homologies to endopeptidases on X chromosome) whose role has been highlighted in hypophosphatemic rickets; however, these initial findings have never been reproduced and this part of FGF23 metabolism remains to be fully understood (10, 11). However, FGF23 can be proteolytically cleaved between Arg^{179} and Ser^{180}, in the Arg^{176}-X-X-Arg^{179} region (4). The active form of FGF23 corresponds to the protein before cleavage, from the 25th to the 251th amino acid; in contrast, the inactive form of FGF23 is obtained after cleavage (11).

2 Animal models and FGF23

Mice knock-out for FGF23 present with a decreased longevity, in association with growth retardation, skin atrophy, decreased bone density and ectopic as well as vascular calcifications. In addition, they present with hyper-phosphatemia, hypercalcemia and increased serum levels of 1,25 dihydroxy vitamin D (12). These mice also have a trend toward an increased sensitivity to insulin and are therefore at increased risk of hypoglycemia. In these animals, a diet with low phosphate intake can improve the clinical phenotype while correcting serum phosphate levels (but without modifying serum calcium and 1,25 dihydroxy vitamin D levels). Similarly, a diet with low native vitamin D can correct serum calcium and 1,25 dihydroxy vitamin D levels (but without modifying serum phosphate levels) as well as improve life expectancy (13).

In contrast, mice over-expressing FGF23 present with a clinical phenotype of hypophosphatemic rickets, with hypophosphatemia, increased phosphaturia and hyperparathyroidism; in these animals serum calcium, 1,25 dihydroxy vitamin D circulating levels and renal function are usually normal (14). However, the renal expression of some of the regulators of phosphate reabsorption is modified, with decreased Klotho and Npt2a for example (14).

3 Klotho: structural properties, overview on its role in physiology and mineral metabolism

Through alternative splicing or ectodomain shedding of the transmembrane protein, the Klotho protein exists both as a secreted and a membrane protein whose extracellular domain could be shed from the cell surface by secretases and released into the circulation to act as an endocrine factor (15).

Membrane Klotho is a single-pass trans-membrane anti-aging protein (1014 amino-acids, 130 kDa, chromosomic location 13q12 in humans) that functions as a coreceptor for FGF (16). Its expression occurs mainly in the kidney and in the parathyroid. Its extra-cellular domain is wide whereas its intra-cellular part is very short.

In contrast, soluble Klotho (also known as α-Klotho) is a multifunction protein present in the biological fluids including blood, urine and cerebrospinal fluid and playing important roles in antiaging, energy metabolism, inhibition of Wnt signaling, antioxidation (through an increased endothelial nitric oxide production), resistance to lipid peroxidation, modulation of ion transport and control of PTH and vitamin D (15).

Mice over-expressing Klotho have an increased life expectancy (16). In contrast, mice lacking Klotho expression present with a decreased life span, with skin atrophy, decreased bone density, ectopic calcifications and infertility. In addition, they present with hyperphosphatemia, hypercalcemia and increased serum levels of 1,25 dihydroxy vitamin D (17). A diet with low native vitamin D can improve the whole phenotype (17). These similarities between mice knock-out for Klotho and FGF23 have led to the description of the fundamental interplay between these two players of phosphate / calcium metabolism.

Klotho is indeed an essential player for FGF23 biological activity, at least for its 'classical' effects. While FGF23 binds with a modest affinity to multiple receptors belonging to the family of FGF receptors (FGF-R, mainly type 1,3 and 4), Klotho seems required *in vivo* for FGF23-mediated receptor activation, thus stimulating the phosphorylation pathways downstream the receptor (18). The highest expression of Klotho/FGF-R complex is in the distal tubule whereas the major biologic effects of FGF23 are located in the proximal tubule (19); this discrepancy is still not explained, but other reports have also described an expression of Klotho in the proximal tubule, with a direct phosphaturic effect only by itself (20). The association between Klotho and FGF23 is stabilized in vitro by heparin, but its potential impact in human physiology has not been evaluated. However, FGF23 does not require Klotho in all models; indeed Klotho is not expressed in bone cells on one hand, and the effects of FGF23 are Klotho-independent in cardiomyocytes on the other hand (21).
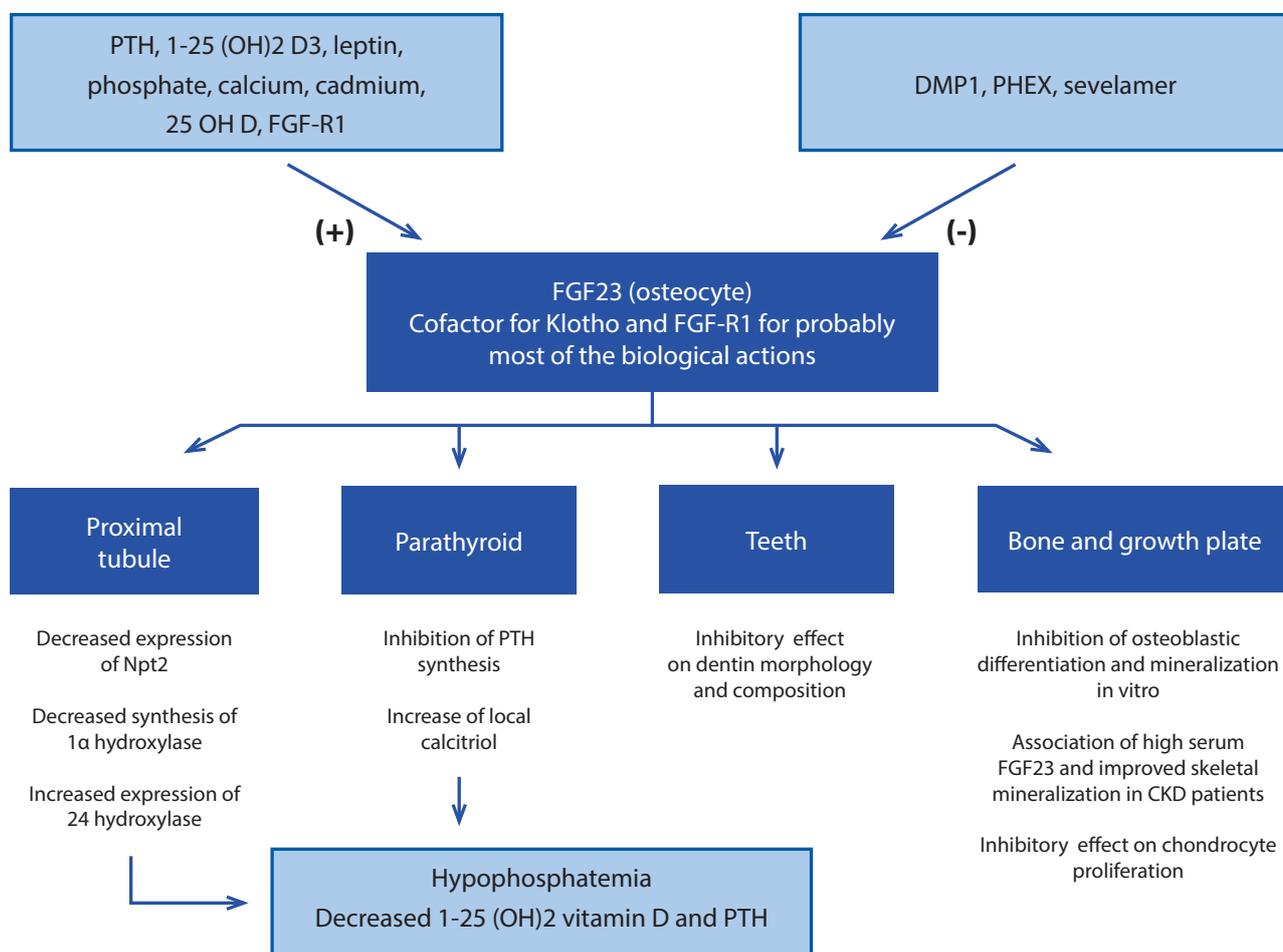
But Klotho also has its own role in the regulation of phosphate/calcium metabolism: it can function as an enzyme modifying the sugar chains of transient receptor potential vanilloid type 5 (TRPV5) in the distal tubule, preventing the calcium channel from internalization and inactivation, and thus leading to increased calcium reabsorption (22). In such a case, Klotho has a β-glucuronidase activity. Klotho can also directly regulate PTH synthesis: when intra-cellular calcium decreases in the parathyroid, the local expression of Klotho increases, therefore inducing an increased activity of the Na-K-ATPase channel, an increased PTH synthesis and a further correction of the hypocalcemic state (23).

Last, apart from its key role in phosphate/calcium metabolism regulation, Klotho has also many other roles in general metabolism. Klotho can regulate other ionic channels, such as the potassium channel ROMK1 (Renal Outer Medullary Potassium channel). In this later setting, its action is similar to the one observed with TRPV5: through a β-glucuronidase activity, it can stimulate urinary excretion of potassium (16). Fischer et al. have also shown that mice with Klotho deficiency have a decreased extra-cellular volume with hyperaldosteronism ; however of interest, the underlying molecular pathways remain to be proven (24). In terms of general metabolism, Klotho resists oxidative stress (through an inhibition of the IGF1 / insulin axis), modulates the Wnt pathway (16), and apoptosis through anti-apoptotic properties (25).

Although its expression is mainly located in the parathyroid and the kidney, an expression of Klotho has also been detected in other tissues, namely choroid plexus, placenta, endocrine organs such as testis, pancreas and ovary, and sinoatrial node: it is therefore likely that other new roles for Klotho will be high-lighted in the future (16). As such, α-Klotho levels were recently shown to be lower among mothers who deliver a small-for-gestational-age neonate than in those with normal pregnancies (26), and Klotho levels in the cerebrospinal fluid of patients suffering from Alzheimer's disease have been shown to be lower than in controls (27).

4   FGF23 in Human Physiology

The figure below summarizes the known roles of FGF23 in human physiology in 2013, from a 'phosphate and calcium metabolism' point of view (28-31).

PTH, 1-25 (OH)2 D3, leptin, phosphate, calcium, cadmium, 25 OH D, FGF-R1

DMP1, PHEX, sevelamer

(+)

(-)

FGF23 (osteocyte)
Cofactor for Klotho and FGF-R1 for probably most of the biological actions

Proximal tubule

Decreased expression of Npt2

Decreased synthesis of 1α hydroxylase

Increased expression of 24 hydroxylase

Parathyroid

Inhibition of PTH synthesis

Increase of local calcitriol

Teeth

Inhibitory effect on dentin morphology and composition

Bone and growth plate

Inhibition of osteoblastic differentiation and mineralization in vitro

Association of high serum FGF23 and improved skeletal mineralization in CKD patients

Inhibitory effect on chondrocyte proliferation

Hypophosphatemia
Decreased 1-25 (OH)2 vitamin D and PTH

(+) corresponding to a positive effect

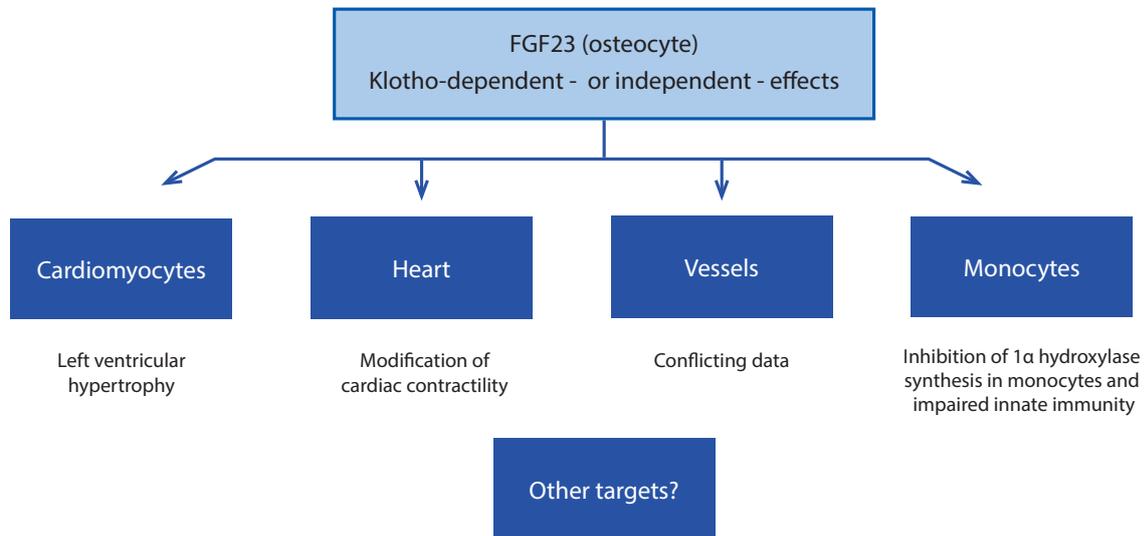*(-) corresponding to an inhibitory effect*

DMP1: dental matrix protein 1

PHEX: phosphate-regulating gene with homologies to endopeptidases on X chromosome

Npt 2: type II sodium-phosphate cotransporters (type a and c)

CKD: chronic kidney disease

The figure below summarizes the known roles of FGF23 in human physiology in 2013, from a more global point of view (21, 32-34).

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                    ┌────────────────────────────────────────┐
                    │          FGF23 (osteocyte)              │
                    │  Klotho-dependent -  or independent - effects │
                    └────────────────────────────────────────┘
        ┌──────────────────┬──────────────┴────────┬──────────────────┐
        ▼                  ▼                        ▼                  ▼
┌──────────────┐   ┌──────────────┐        ┌──────────────┐   ┌──────────────┐
│Cardiomyocytes│   │    Heart     │        │   Vessels    │   │  Monocytes   │
└──────────────┘   └──────────────┘        └──────────────┘   └──────────────┘

 Left ventricular    Modification of        Conflicting data    Inhibition of 1α hydroxylase
   hypertrophy     cardiac contractility                        synthesis in monocytes and
                                                                impaired innate immunity

                   ┌──────────────┐
                   │Other targets?│
                   └──────────────┘
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FGF23 regulation involves both transcriptional and post-translational mechanisms, together with systemic and local bone-derived factors. PTH, vitamin D, phosphate and calcium stimulate FGF23 synthesis whereas glycophosphoproteins synthesized by osteocytes can activate or inhibit FGF23 secretion (e.g., Matrix Extracellular Phosphoglycoprotein MEPE and Dentin Matrix Protein 1 DMP1, respectively) (35). These two proteins are strongly involved in bone mineralization, but MEPE has also been described as a phosphaturic factor by itself in the renal tubule (36). Interestingly, a recent paper also demonstrated that FGFR1 was able to regulate the FGF23 expression, an antibody-mediated activation of FGFR1 being indeed able to induce FGF23 mRNA synthesis and further hypophosphatemia in murine models; siRNA-mediated FGFR1 knockdown induced opposite effects (37).

The role of FGF23 on bone needs further evaluation but studies have demonstrated that FGF23 overexpression *in vitro* can suppress not only osteoblast differentiation but also matrix mineralization, independently of its systemic effect on phosphate metabolism (38), whereas Klotho is not expressed in bone. In contrast, other authors have reported a positive effect on FGF23 on bone: for example, Wesseling-Perry and al. have reported an association between high levels of circulating FGF23 and improved indices of skeletal mineralization (i.e., decreased osteoid thickness and shorter osteoid maturation time) in pediatric patients undergoing peritoneal dialysis (39). In medical conditions such as hypophosphatemic rickets or tumor-induced osteomalacia (TIO), the exact role of FGF23 on bone has not been yet established. A Japanese report has highlighted that patients with *osteogenesis imperfecta* receiving pamidronate infusions present an acute decrease of FGF23 circulating levels, but, again, the underlying mechanisms remain to be demonstrated (40).

5 FGF23 and Klotho in genetic human diseases

The initial description of FGF23 was done in the early 2000's, with studies focusing on hypophosphatemic rickets (41). However, it has rapidly been understood that the spectrum of FGF23 deregulation was wider, with the description of its role in TIO, and then in patients with chronic kidney disease (CKD).

Mutations of FGF23 and most of its regulators have been reported to account for either hypophosphatemic either hyperphosphatemic diseases. The table below summarizes our current knowledge of genetic diseases associated directly or indirectly (through their regulators) to FGF23 and Klotho (5, 41-45).

	DISEASE	INVOLVED GENES
Hypophosphatemia	Hypophosphatemic rickets	Activating mutation of FGF23 *** Inactivating mutation of PHEX *** Inactivating mutation of DMP 1 *** Inactivating mutation of ENPP1 *** Inactivating mutation of Npt2c ** Activating translocation of Klotho * / **
	With renal lithiasis and/or osteopenia and/or hypercalciuria	Inactivating mutation of Npt2a ** Inactivating mutation of Npt2c ** Inactivating mutation of NHERF1
	Mac Cune Albright / fibrous dysplasia of bone	Overexpression of FGF23, GNAS ***
	Tumor induced osteomalacia	Overexpression of FGF23, MEPE, FGF7 and/or FRP4 ***
	Epidermal naevus syndrome	FGFR3 ***
Hyperphosphatemia	Familial tumoral calcinosis	Inactivating mutation of FGF23 ** Inactivating mutation of Klotho *** Inactivating mutation of GALNT3 **

FGF   *fibroblast growth factor*
PHEX   *phosphate-regulating gene with homologies to endopeptidases on the X chromosome*
DMP1   *Dentin matrix protein 1*
ENPP1   *ecto-nucleotide pyrophosphatase / phosphodiesterase 1*
Npt2a   *type IIa sodium-phosphate cotransporter (SLC34A1)*
Npt2c   *type IIc sodium-phosphate cotransporter (SLC34A3)*
MEPE   *matrix extracellular phosphoglycoprotein*
FRP4   *frizzled related protein 4*
FGFR3   *fibroblast growth factor receptor 3*
GALNT3   *UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3*

*   *with associated hyperparathyroidism*
**   *disease associated with a low FGF23 serum level*
***   *disease associated with a high FGF23 serum level*

Briefly, hypophosphatemic rickets correspond to a heterogeneous genetic disease, affecting 1/20 000 children, resulting from mutations in FGF23, Klotho or their regulators to induce hypophosphatemia, rickets, dental abnormalities and bone deformations. Patients present with hypophosphatemia, decreased tubular phosphate reabsorption, normal 25OH vitamin D and PTH, increased alkaline phosphatase. The clinical management combines high oral phosphate intake and active vitamin D therapy, allowing a better growth and correcting the bone deformations, but explaining the two main complications often observed in this disease (i.e., secondary hyperparathyroidism secondary to phosphate intake, and nephrocalcinosis secondary to active vitamin D therapy) (46).

While hypophosphatemic rickets are usually diagnosed during early childhood, an acquired disease with the same phenotype can also affect adults (more exceptionally children or teenagers), i.e., TIO (47). These TIO correspond to mesenchymal tumors, usually benign and located in the appendicular skeleton ; they result from an acquired hypersecretion of phosphatonins (mostly FGF23, sometimes SFRP4, MEPE or FGF7) (48). Patients present with osteomalacia, bone pains, fractures and muscular weakness; a normal phosphate level years or months before the onset of the clinical picture is a strong rationale for an acquired cause of FGF23 hypersecretion. In such a case, the search for the tumor should be performed with accurate tools (tomography, MRI, Tc^{99m} bone scintigraphy, octreotide scintigraphy, F18-fluorodeoxyglucose scan), since these tumors are small and difficult to diagnose in one hand, and since hypophosphatemia will be corrected within days after surgery on the other hand (48).

In contrast, familial tumoral calcinosis is a rare autosomal recessive disorder due to inactivating mutations of FGF23, Klotho, GALTN3 or SAMD9 (49, 50). Patients present before the age of 20 with soft tissues calcifications around joints, in association with vascular calcifications and sometimes hyperostosis. Biologically, hyperphosphatemia, normal circulating calcium, increased 1-25 OH$_2$ vitamin D and decreased PTH levels are observed. Management is challenging, aiming at decreasing intestinal absorption of phosphorus (with sevelamer or aluminum-derived phosphate binders for example) and/or increasing urine phosphate (acetazolamide or probenecide).

6   FGF23 and Klotho deregulation during chronic kidney disease

FGF23 metabolism is strongly modified by CKD, and most studies have focused on this deregulation: FGF23 increases as GFR decreases, from early stages of CKD, even before serum phosphate and PTH have become abnormal (51, 52).

In healthy volunteers, oral phosphate loading stimulates FGF23 synthesis while it is the contrary for dietary phosphate restriction (53). FGF23 levels increase progressively as GFR decreases, some papers reporting significant increases already by stage 2 and 3, i.e., well before the onset of a critical reduction in the nephron number (54, 55). Although many studies have clearly shown the deregulation of FGF23 metabolism in the advanced stages of CKD, the mechanisms by which FGF23 serum levels increase during the early stages of CKD, even before serum phosphate or PTH increase or 1-25 vitamin D decreases, remain mysterious (56); current hypotheses propose increased FGF23 as the earliest alteration in mineral metabolism in CKD, resulting from an increased bone production rather than a decreased renal clearance (52, 54, 55). This increase could be explained by different factors, such as:

1. a decreased clearance of FGF23,
2. a compensatory mechanism in an attempt to excrete the excess serum phosphate and keep serum phosphate within the normal range,
3. a response to the treatment with active vitamin D analogs,
4. a compensatory mechanism to the loss of the kidney-secreted Klotho protein, but it is not clear whether the biological effects of FGF23 are increased or decreased in this situation, or
5. an increased production of FGF23 in bone cells (5, 42). Pereira et al. have well demonstrated in 32 children and young adults with CKD stage 2-5 that the expression of FGF23 in bone was increased at all stages of CKD, with a positive association between bone expression and circulating levels of FGF23 (31), therefore giving strength to the increased bone production hypothesis. However, the trigger of this over-production remains to be determined…

During the course of CKD, serum FGF23 levels are positively correlated with serum phosphate and negatively with serum calcitriol and PTH. The role of FGF23 to explain the onset of hyperparathyroidism can be explained by different and various direct and indirect effects. First, since FGF23 has a counter-regulatory effect on vitamin D, the increased FGF23 during CKD has the potential to reduce vitamin D activity, and thus to facilitate the development of secondary hyperparathyroidism (5). Second, FGF23 can also stimulate the local expression of 1α hydroxylase in the parathyroid, suggesting that it could also indirectly down regulate PTH synthesis through an increased local production of calcitriol (7). Last, it has been well demonstrated both in CKD rats, in dialysis patients, and in CKD patients (among them some patients with a past of renal transplant) that there was a down-regulation of the FGF23 signaling pathway in the parathyroid glands with:

1. a decreased expression of FGFR1 and Klotho in parathyroid cells, and
2. a resistance to FGF23 administration in CKD rats, i.e., the absence of decreased PTH synthesis (57-60).

All these observations can therefore explain, at least partly, the refractory secondary hyperparathyroidism observed in CKD patients.

As detailed above, there is an accumulation of FGF23 in CKD. However, it remains questionable whether this accumulation corresponds to active or inactive fragments. In a small series of 14 end-stage renal disease (ESRD) adult patients, Weber et al. initially demonstrated an accumulation of C-term FGF23 fragments, suggesting that less than one quarter of the circulating FGF23 was bioactive in patients with end-stage renal disease, but recent data strongly support the conclusion that all circulating FGF23 in children and adults undergoing peritoneal dialysis is intact and biologically active (61). While several authors have discussed a modification of the intact/C-terminal FGF23 ratio in CKD adults (62), this ratio does not seem useless in all CKD populations (63).

In parallel to this early increase of FGF23 levels, Klotho circulating levels also decrease early during CKD (64, 65). Indeed, in this cross-sectional study of 87 CKD stage 1-5 patients, Klotho linearly decreased, whereas FGF23 showed a baseline at early CKD stages and then a curvilinear increase. Crude mean Klotho level declined by 4.8 pg/mL (95% CI 3.5-6.2 pg/mL, P < 0.0001) as GFR declined by 1 mL/min/1.73 m^2 (65). These human data were further confirmed in murine models, demonstrating a decreased renal expression of Klotho in mice with CKD (66). Moreover, Klotho polymorphisms have been associated to overall prognosis in this population: one specific Klotho polymorphism could be a protective factor against CKD progression of non-diabetic ESRD in African Americans (67), while another Klotho specific variant could increase global mortality at one year (68), and another one has been found to be associated with increased uric acid levels and decreased low density lipoprotein cholesterol in hemodialysis patients (69). Interestingly, this effect was even more marked in patients not receiving active vitamin D supplementation, keeping in mind that vitamin D is a positive regulator of Klotho (70), through the activation of a VDRE directly in the Klotho promoter. The degree of methylation of the Klotho promoter has also been shown to be associated with the severity of CKD in a cohort of Chinese patients (71). Last, in the kidney, the erythropoietin receptor and its activity are downstream effectors of Klotho enabling it to function as a cytoprotective protein against oxidative injury; these promising murine data could be of interest in the context of CKD-induced anemia (72).

All this deregulation of FGF23/Klotho are now well described in the context of chronic kidney disease, but recent data have also demonstrated an early significant increase of FGF23 levels during acute kidney injury (AKI) both in humans and in rodent models (73). In addition to this early deregulation of FGF23 in AKI, Klotho also has protective renal effects in a specific AKI model (namely cisplatin-induced AKI), through a decrease of basolateral uptake of cisplatin on one hand, and through an anti-apoptotic effect on the other hand, this later being cisplatin-independent (74).

7 FGF23, Klotho and the cardiovascular system

In general populations, Parker et al. have described an increased risk of mortality and cardiovascular events in patients with stable coronary disease and greater FGF23 levels (75), while elevated serum FGF23 levels, even within the normal range, are associated with increased left ventricular mass index and increased risk for the presence of left ventricular hypertrophy in a cohort of 795 Swedish elderly subjects (76).Recent papers also demonstrated that baseline FGF23 is a predictor of the severity of coronary artery disease in general populations (77), and that FGF23 is a predictor of severe abdominal aortic calcifications (33).

CKD patients often present with a phenotype close to the one observed in Klotho or FGF23 deficient mice, i.e., hyperphosphatemia, ectopic calcifications, vascular calcifications, hypogonadism and premature death (78). In that setting, circulating FGF23 levels can also provide prognostic information in CKD patients, mainly in terms of CKD progression, therapeutic response and cardio-vascular mortality. In a prospective cohort of 177 non diabetic CKD patients, FGF23 was an independent predictor of CKD progression, with a cut-off serum level of 104 RU/mL at inclusion for the C-term assay (79). In a prospective study of 62 dialysis patients, baseline intact FGF23 levels in association with baseline PTH serum levels were found to be good predictors of refractoriness to intravenous calcitriol therapy at 24 weeks (80). Moreover, baseline intact FGF23 serum level (cut-off 7500 ng/L) was described as a potential predictor at two years of refractory hyperparathyroidism in 103 non diabetic dialysis patients with mild hyperparathyroidism at baseline (81). Gutierrez et al. demonstrated that higher quartiles of serum FGF23 were associated with an increased risk of mortality in hemodialysis adult patients (82); Jean et al. obtained similar results in a cohort of 219 hemodialysis patients, with an increased risk of mortality and vascular calcifications in patients with higher quartiles of FGF23 serum levels two years after inclusion (83).

Recently, after these epidemiological data showing a relationship between FGF23 and cardiovascular outcomes, another step in the understanding of this pathway was made, with the first description of an off-target of FGF23 in cardiomyocytes. Indeed, Faul et al showed that FGF23 could regulate cardiomyocytes' biology in a Klotho-independent manner (21). After a clinical demonstration that the left ventricular mass index was significantly increasing with higher quartiles of circulating FGF23 levels in a prospective cohort of 3 070 adults with CKD from the CRIC study and that greater levels of FGF23 levels at baseline were also associated with a higher risk of new-onset left ventricular hypertrophy (LVH) in CKD, the authors studied the direct effects of FGF23 in cellular (cardiomyocytes from rats) and animal (wild-type mice, Klotho deficient mice and rats with 5/6th nephrectomy) models. They showed that FGF23 was able to induce *in vitro* hypertrophy of cardiomyocytes, with an activation of prohypertrophic genes; this effect was dependent of the activation of the FGF-receptors (and notably FGF-R1 and 4). Of note, in contrast to renal or parathyroid cells, these neonatal cardiomyocytes did not express Klotho. Moreover, the main downstream phosphorylation pathway involved in this activation was the PLCγ-calcineurin-NFAT axis (that can be inhibited by the use of cyclosporine), whereas in renal and parathyroid cells exposed to FGF23, the activated pathways are usually rather Erk and Akt (84). After this cellular demonstration of a direct deleterious effect of FGF23 on cardiac cells, the authors delivered FGF23 in wild-type mice either through a direct myocardial delivery of FGF23 either through an intravenous infusion for 5 days, showing in both conditions the induction of LVH. In a genetic mouse model of high FGF23 circulating levels (i.e., the Klotho deficient mice), they also showed that these mice developed LVH, therefore providing another rationale for a Klotho-independent effect of FGF23 in the cardiovascular system. Last, using a rat model of CKD (5/6th nephrectomy), they showed that a systemic treatment of these animals with the FGFR inhibitor PD173074 was able to attenuate the development of LVH, despite having no effects on the severity of CKD or arterial hypertension. More recently, Touchberry et al showed that FGF23 could also induce an elevation of intracellular calcium in cardiomyocytes, in addition to an increase of ventricular muscle strip contractility; these effects could be blocked using the L-type Ca channel blocker verapamil (32).

Thus, all these data provide a mechanistic explanation and demonstrate that FGF23 effects can be Klotho-independent, thus providing a strong rationale for future clinical trials of FGF23 monoclonal antibodies.

Taken together, all these data from epidemiological and basic studies demonstrate the presence of an association between FGF23 and left cardiac hypertrophy.

In the setting of vascular calcifications (VC), the role of FGF23 is less clear. Indeed, in the recent years of the 'FGF23 story', it was thought to be a protective factor against VC (85), but many data have challenged this initial observation. Indeed, severe calcification aortic scores (CAC) have been reported to be associated with higher FGF23 levels in non-dialysis and hemodialysis patients (86, 87), but these associations did not remain systematically significant by multivariable analyses. Scialla et al have clearly demonstrated that higher quartiles of FGF23 in CKD patients were not associated with VC while higher quartiles of phosphate were significantly associated with VC (88).

Recent experimental studies support a role for FGF23 and its co-receptor Klotho in cardiovascular pathology, yet the underlying mechanisms remain largely elusive. Klotho deficiency causes and enhances VC in murine models of CKD; indeed, Hu et al demonstrated in a model of murine CKD very low circulating and renal levels of Klotho, but CKD transgenic mice overexpressing Klotho presented with a better renal function and much less VC compared with wild-type mice with CKD (89). In a cohort of 114 CKD patients, decreased serum Klotho levels have been shown to be an independent biomarker associated with arterial stiffness; in this study Klotho levels correlated directly with 1-25 vitamin D and inversely with PTH (90).

Directly in the vessels, Klotho, FGF-R1 and FGF-R3 appear to be expressed in human normal arteries; human smooth muscle cells (normally expressing Klotho) cultured with FGF23 up-regulate the downstream kinases p-ERK and p-AKT (91). Lim et al. also described endogenous Klotho expression in human arteries and human aortic smooth muscle cells; through mechanistic studies, these authors demonstrated that Klotho knockdown potentiated the development of accelerated calcification through a Runx2 and myocardin-serum response factor-dependent pathway. Moreover, vascular cells are Klotho-dependent for FGF-23 effects since FGF23 induced the activation of p-ERK and p-AKT, which were abrogated following Klotho knockdown. Last, vascular Klotho deficiency could be restored using vitamin D receptor activators. Therefore, they described a double role for local vascular Klotho: an endogenous inhibitor of vascular calcification and a cofactor required for vascular FGF-23 signaling (92). However, Lindberg et al obtained inverse results in murine arteries from wild-type mice and from a novel mouse model harboring a vascular smooth muscle cell specific deletion of Klotho. Klotho protein levels were undetectable by immunohistochemistry and Western blots in the arteries of the two murine models. Intravenous infusion of FGF23 induced a rise in renal but not arterial Egr-1 expression, a marker of Klotho-dependent FGF23 signaling. In bovine vascular smooth muscle cells (bVSMC), the same authors demonstrated that FGF23 did not modify calcification in bVSMCs. These data therefore do not support Klotho-mediated FGF23 effects in the vasculature (93). Last, other effects of Klotho in vessels have been described such as the restoration of vasodilation capacities through endothelium-derived NO production (94), and the maintenance of endothelial integrity (95).

In the heart, Klotho is not expressed, except in the sino-atrial node where it is essential for electric activity (96). Even though there is no expression of Klotho in the heart, Klotho displays cardioprotective effects that are mediated by a downregulation of TRPC6 channels (97). Last, it is also interesting to note that an activation of the renin angiotensin system will decrease the renal expression of Klotho while the use of ACEi or ARBs will upregulate tissular Klotho (98).

In conclusion, many conflicting data have been published on the potential role of FGF23 and Klotho in heart and vessels, but FGF23 appears to be a deleterious actor in the heart while Klotho appears to have beneficial effects both in the heart and in the vessels. In the future, the next steps would be to confirm that strategies lowering FGF23 levels or increasing Klotho levels can also decrease cardiovascular and overall mortality in CKD patients; however, after the recent results published in rodents leading to increased mortality rates in CKD animals receiving the monoclonal FGF23 antibody, physicians may stay cautious (99).

8 FGF23, Klotho and global health

In terms of bone status and fracture risk, data are conflicting in the literature: some authors showed, for example in a prospective cohort of 2 868 men (75±3 years, median follow-up 3.4 years), that baseline FGF23 levels were directly correlated to the overall fracture risk, with the strongest relation when FGF23 was above 56 pg/mL; this relationship remained after adjustment on all other fracture risk factors (100). However, recent series displayed conflicting data: in the cardiovascular health study for example (corresponding to a cohort of 2 008 women and 1 329 men older than 65 years of age), greater FGF23 levels were associated with greater bone densities assessed by DXA, with no association between FGF23 and the risk of fracture (101).

From a more metabolic point of view, other studies showed that FGF23 circulating levels were also negatively associated with high-density lipoprotein and apolipoprotein A1 and positively with triglycerides. FGF23 levels were higher in subjects with metabolic syndrome compared with those without (102). In pediatrics, FGF23 levels were also associated with BMI (103).

In humans, Klotho polymorphisms have been associated both in a positive and negative way to bone mineral density, life expectancy, cardiovascular events (e.g., ischemic stroke, carotid atherosclerosis), biomarkers of metabolic syndrome (e.g., uric acid levels, lipid and glucose metabolisms), kidney stones, and even to cognitive ability (104-110).

9 Different assays for measuring circulating FGF23 and Kotho

9.1 FGF23

Following the description of an active and an inactive form of FGF23, **different assays** have been developed for serum FGF23 measurement: the 'intact' assay that measures only active FGF23 and the 'C-terminal' assay that measures both the active and inactive FGF23. The figure below summarizes the two different types of assays for measuring FGF23 concentrations.
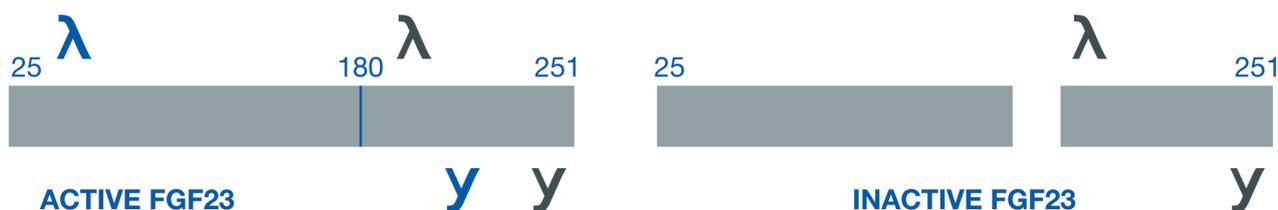
*in blue, antibodies used to measure only the active FGF23: **intact assay***
*in gray, antibodies used to measure both the active and the inactive FGF23: **C-terminal assay***

At least three studies have well demonstrated the absence of circadian intra-individual variability for FGF23 (63, 111, 112). Racial differences could account for differences in FGF23 circulating levels: for example, in a cohort of 1 099 CKD patients undergoing conservative therapies, it has been well showed after adjustment that African American patients had decreased FGF23 and 25OH vitamin D levels and increased PTH levels (113). In the same time, FGF23 levels are greater in women, in persons with tobacco exposure and diabetes (75). It remains questionable whether factors such systemic inflammation, infection or therapies can influence FGF23 serum levels by themselves; however, body weight (positive association between BMI and serum FGF23 levels), a past of solid organ transplantation as well as a corticosteroids treatment appear to influence FGF23 circulating levels (63).

Even though the manufacturers do not provide reference values, highlighting the fact that these assays should be used only for research purposes, reference values have nevertheless been proposed in healthy population: **in adults**, the reference values for C-terminal FGF23 are 55±50 RU/ml (111), and 10 to 50 pg/mL for intact FGF23 (114). There are very few **pediatric data** on FGF23. An initial study reported normal values of C-terminal FGF23 serum levels of 69±36 RU/mL in 26 children (10.9 ± 5.5 years), however without specific data confirming normal renal function

(111); since this initial report, other teams have proposed reference values according to age, gender and glomerular filtration rate with the first generation C-terminal assay and the intact one (63): the table below summarizes the reference values (results expressed as mean with 95% confidence interval) for C-terminal and intact FGF23 according to age in the 115 children with a normal renal function (i.e., glomerular filtration rate between 90 and 139 mL/min per 1.73 m^2). Of note, gender did not influence the results in this pediatric population, in contrast to the Heart and Soul study (63, 75).

Age (years)	5-10	10-15	15-20
N	57	33	25
C terminal FGF23 (RU/mL)	65 (39-91)	49 (25-73)	76 (6-146)
Intact FGF23 (pg/mL)	32 (28-37)	36 (30-41)	45 (37-54)

In younger children, Brown et al. described an elevated FGF23 serum level in a child with Jansen's metaphyseal chondrodysplasia in comparison to 5 healthy children aged 10-18 months; in that study, reference FGF23 serum levels were 30±17 pg/mL with an intact assay, and 104±36 RU/mL with a C-terminal assay (115). Other papers have also studied FGF23 levels in newborns, showing a rapid decrease of the intact / C-term ratio during the first 5 days after birth (116).

In conclusion, FGF23 circulating levels on EDTA plasma can now be measured with immunometric assays that detect either the intact hormone alone or either the intact FGF23 as well as the C-terminal fragments; it seems preferable to use an intact FGF23 assay to establish the diagnosis of FGF23-dependent hypophosphatemic disorders (55).

9.2   Klotho

It is possible to measure α-Klotho circulating levels in human blood (112, 117). However, by definition, this kit measures the portion of α-Klotho that is circulating in the systemic circulation after its release from the cell membrane after shedding. The clinical relevance of such a circulating α-Klotho remains to be fully understood, since the main biological effects of Klotho are probably mediated by the trans-membrane form of the protein.

In contrast to the stability of FGF23 circulating levels among time, Klotho has a circadian rhythm, with a nadir around midnight and a maximum concentration in the early morning (112). Yamazaki et al. have reported reference serum α-Klotho values in 142 healthy volunteers (66 men, 61±19 years, serum creatinine 61±12 μmol/L): the values ranged from 239 to 1266 pg/mL (mean±SD: 562±146 pg/mL). The levels of α-Klotho were not influenced by gender or indices of skeletal metabolism, but were inversely related to serum creatinine and age. Moreover, in that study, Klotho seems to decrease with age (112, 117) and the additional analysis of 39 Asiatic children (23 boys, age 7±5 years; serum creatinine 32±11 μmol/L) showed that the concentration of the soluble form of α-Klotho was 952±282 pg/mL (i.e., significantly greater than in adults), with an intact FGF23 of 24±12 pg/mL. Analyses including both adults and children showed a positive relationship between α-Klotho and serum phosphate, whereas the association was inverse between α-Klotho and both FGF23, calcium, creatinine and age (117). Recently, Ohata et al. have also showed that the levels of alpha-Klotho were markedly higher in cord blood than in neonates at four days of life, in mothers and adult volunteers, while the fetal levels of FGF23 were lower; the levels of soluble alpha-Klotho and FGF23 were inversely correlated in cord blood (118).

In conclusion, α-Klotho circulating levels on EDTA plasma or serum can now be measured with immunometric assays but their use should be restricted for research use in 2014.

10 When should we measure FGF23 levels in clinical practice?

Before answering this question, the first question could be: should we measure FGF23 levels in clinical practice? With the second-generation assay and its better accuracy, the technique is robust but unfortunately it is not available everywhere. Two situations could theorically benefit from FGF23 measurements: CKD-related diseases, and non-CKD diseases.

First, in non-CKD diseases, such as hypophosphatemic rickets/TIO and familial tumoral calcinosis, it could be interesting to measure FGF23 levels at the time of diagnosis; in contrast, the meaningfulness of monitoring FGF23 levels in patients with hypophosphatemic rickets receiving active vitamin D analogs and oral phosphate supplementation remains debatable since it has been shown that FGF23 levels increase with therapy, and that the alkaline phosphatase levels are a good marker of efficacy (119).

Second, in CKD, additional studies are warranted to determine whether a target of FGF23 could be proposed to adapt therapies, in addition to the calcium, phosphorus and PTH levels recommended in international guidelines depending on the different CKD stages. To date, the follow-up and drug modifications should not depend on FGF23 levels. In dialysis patients, it is probably too late to monitor FGF23 levels, it may be interesting in early CKD, but, again, simple markers of compliance are also available (such as 24-hours phosphaturia).

11 Hot topics in research

Even though many questions remain unsolved (54), FGF23 and Klotho have obviously modified our understanding of phosphate / calcium metabolism, and a therapeutic targeting of this axis will probably be the next step, at least in CKD patients. Economic and public health consequences of such strategies can be important, since the prevalence of CKD is growing worldwide, as well as the number of patients reaching ESRD.

FGF23 is a negative predictor of survival and cardio-vascular morbidity in different sub-groups of patients, but its direct toxicity has been demonstrated only in few *in vitro* models (21). However, even though it remains debatable whether FGF23 is a direct culprit or an innocent bystander (120), **strategies aiming at decreasing FGF23 levels or at restoring Klotho circulating levels could be of interest in CKD** (5, 9). In such a setting, FGF23 could also represent a biomarker to adapt all the therapies aiming at controlling CKD-MBD. Some trials have reported a decrease of FGF23 levels:
1. in early stages of CKD in adults receiving the non-calcium phosphate binder sevelamer (121),
2. in early stages of CKD in adults lowering dietary phosphate intake (122), and
3. in hemodialysis and peritoneal dialysis patients receiving the calcimimetic cinacalcet (123,124).

Moreover, a post-hoc analysis of a one-year randomized trial of phosphate binders (sevelamer vs. calcium acetate, with or without calcitriol) in 72 hemodialysis patients has showed that a more significant FGF23 decrease was obtained in patients taking sevelamer, not receiving calcitriol, and on a 2.5 mEq/l calcium dialysate (125). Other trials evaluating the effect of other non-calcium phosphate binders (i.e., lanthanum carbonate) or calcium carbonate in early CKD stages have not showed a decrease of FGF23 levels (121, 126), but a recent paper has highlighted decreased FGF23 levels in 18 patients with early CKD (stage 3) receiving lanthanum carbonate for 4 weeks (127). Moreover, in rat models of early CKD, antibodies directed against FGF23 decrease PTH levels, increase calcium and phosphate levels, and normalize 1-25 vitamin D levels (128). In healthy volunteers, native vitamin D supplementation with ergocalciferol increases FGF23 levels (129).

Will all these data in mind, the picture is nevertheless not complete, and one should not forget the results published in 2012 showing in a rat model of CKD that FGF23 neutralization using a monoclonal FGF23 antibody was able to improve the CKD-associated hyperparathyroidism by decreasing PTH levels, increasing vitamin D levels and

normalizing bone biomarkers; however, this drug increased serum phosphate, aortic calcifications and eventually the global mortality of these rats (99). This paper highlighted that we should keep in mind that the line between adaptation and maladaptation is really thin in chronic diseases (130), but also in general populations, since in healthy volunteers the administration of native vitamin D, that is commonly seen as a positive action on global health, is able to significantly increase FGF23 circulating levels (129)…

In a pediatric population, strategies aiming at decreasing FGF23 levels could also be of interest for patients with **hypophosphatemic rickets**. In a mouse model of this model (Hyp mice), the administration of specific antibodies directed against FGF23 led to an improvement of the phenotype (131, 132); human clinical trials are on-going but results are not yet available.

Recently, a link between **iron metabolism and FGF23** has also been discussed: interestingly, iron infusions (often used in CKD and dialysis patients to treat anemia in addition to erythropoietin-stimulating agents) have been showed to induce hypophosphatemia, and to increase FGF23 in patients with iron deficiency and normal renal function (133, 134). In CKD patients, one report was published, with a life-threatening hypophosphatemia following iron infusion in a woman with a past of renal transplantation (135). The clinical implications of such observations and the underlying molecular mechanisms remain to be determined, but the overall of systemic inflammation on FGF23 levels could probably be discussed. In that setting, iron deficiency may stimulate FGF23 transcription or allow FGF23 stabilization. Indeed, there is an inverse association between C-terminal FGF23 and serum iron in healthy individuals, patients with hypophosphatemic rickets and wild-type mice. Although there is also an inverse relationship between intact FGF23 and serum iron in patients with autosomal dominant hypophosphatemic rickets (ADHR, due to a mutation in the FGF23 gene leading to a resistance of FGF23 to cleavage, and thus to an accumulation of the active form of the FGF23), this later association was not found in healthy individuals, therefore leading to the hypothesis that iron could be involved in the cleavage of FGF23, but underlying molecular mechanisms beyond this phenomenon are completely unknown to date (136). Moreover, in ADHR, the human phenotype is heterogeneous, from completely unaffected patients to patients presenting with a delayed onset of the clinical hypophosphatemic phenotype; in a murine model of ADHR corresponding to a knock-in mouse with the mutation involved in FGF23 stability, it has been recently demonstrated that a low iron diet can induce the complete phenotype whereas the mice with the mutation and a normal diet were completely normal, these results thus highlighting a new example of a gene/environment interaction (137). Last, abstracts at the 2010 and 2011 American Society of Nephrology conferences as well as studies found epidemiological links between hemoglobin levels and FGF23 in CKD and in healthy persons (138).

Another point of interest would be **to determine whether FGF23 could be synthesized by other cell types in case of specific diseases;** in addition to demonstrate that baseline FGF23 levels were a predictor of mortality in patients with end-stage liver disease, it was also shown that liver cells from mice with liver lesions were able to directly express FGF23 (139). A similar mechanism could be hypothesized in patients with autosomal dominant polycystic kidney disease (ADPKD), since human data have shown that FGF23 increases in adult patients with ADPKD while circulating Klotho levels decrease, even when renal function is normal (140, 141). When comparing ADPKD patients to healthy volunteers, there was no difference for PTH, phosphorus and TmP/GFR, whereas when comparing ADPKD patients to non-diabetic CKD patients, there was no difference for PTH and TmP/GFR but a significant increase of FGF23 circulating levels and a significant decrease of phosphorus levels. With these data in mind, these authors suggest a resistance to the phosphaturic effect of FGF23 in ADPKD that could be explained through the decreased Klotho levels, but the fact that serum phosphate seemed to be lower in ADPKD patients than in healthy volunteers weakens this hypothesis. Another explanation, namely a down regulation of Klotho by increased FGF23 could also be discussed, as already shown in murine models of overexpression of FGF23 (142). Nevertheless, alternative hypotheses could be either a deregulation of FGF23 by osteocytes that could be triggered by a defect in the primary cilium complex, or an ectopic secretion of FGF23 by the tubular cells (143).

In addition to these human and animal therapeutic trials, **the underlying molecular mechanisms and cellular targets of FGF23 remain to be more accurately identified,** while Klotho is also by itself a systemic hormone with systemic and very various effects.

Other biomarkers synthesized by the 'amazing' osteocyte such as **sclerostin** are now widely recognized as other key factors for bone and mineral homeostasis(144), and probably also vascular calcifications ; in that setting, the recent description of a cross-regulation of vitamin D and FGF23 metabolism by sclerostin brings a new level of complexity in all this physiological mechanisms (145), but also in CKD (146, 147)…

12   Conclusion

In one decade, the description of the key role of FGF23 and Klotho in the 'parathyroid-bone-kidney' axis had led to a better understanding of genetic conditions associated with hypophosphatemia and phosphate calcium disorders as well as CKD-MBD. However, although a therapeutic targeting of this pathway seems likely in the future, many questions remain unresolved and conflicting; the availability of FGF23 and Klotho assays in daily practice for bench and clinical research will allow us in a next future to better understand this fascinating and global pathway.

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FGF-23 Intact, Human 2nd Generation
FIBROBLAST GROWTH FACTOR 23

	Intact
Cat. No.	60 - 6600
Tests	96
Method	ELISA
Range	20 - 660 pg/ml (can be extended to 2200 pg/ml)

Sensitivity	1.5 pg/ml
Incubation time	3.5 hours
Sample volume	50 µl

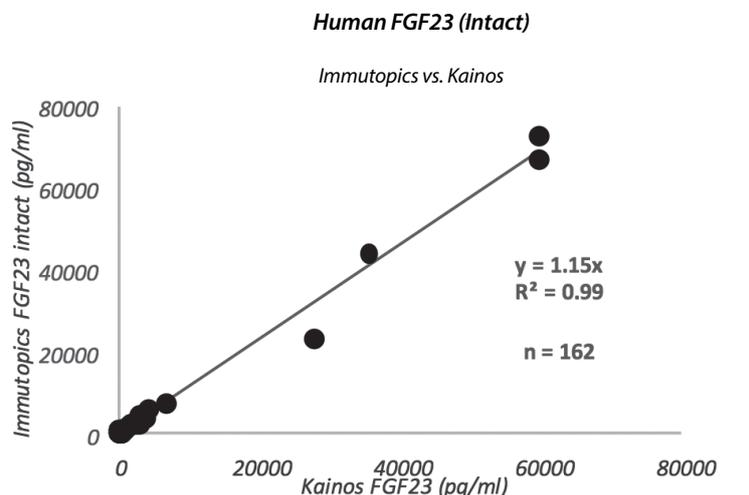|	
Sample type	EDTA & Heparin plasma, cell culture
Sample preparation	It is recommended to collect the sample in the morning after a 12-hour fasting period. Intact FGF-23 is very instable. Therefore, collection and testing or storage should take place promptly. Store samples at -20 °C or below. Avoid repeated freezing and thawing of specimens.

Reference values

Mean: 40.7 pg/ml, SD 16.1 pg/ml
Range: 8.6 - 72.8 pg/ml +/- 2 SD

Species Human

Specificity Antibodies bind to NH2-terminal and C-terminal epitopes; measures only the intact form of the molecule.

Correlation

Human FGF23 (Intact)

Immutopics vs. Kainos

y = 1.15x
R² = 0.99

n = 162

FGF-23 (C-Term) 2nd Generation

FIBROBLAST GROWTH FACTOR 23

|	
C-terminal	
Cat. No.	60 - 6100
Tests	96
Method	
Range	1.5 - 1500 RU/ml
Sensitivity	1.5 RU/ml
Incubation time	3.5 hours
Sample volume	100 µl
Sample type	Heparin- and EDTA Plasma, cell culture
Sample preparation	It is recommended to collect the sample in the morning after a 12-hour fasting period. Keep sample frozen at -20°C. Longer storage at -80°C. Avoid repeated freeze/thaw cycles.

Reference values

	Range (RU/ml)
Premenopausal women (<40 years)	20.9 – 91.1
Postmenopausal women (>60 years)	44.0 – 139.9
Men (27 – 76 years)	33.7 – 96.5

Children:

Group Age	n	Mean (RU/ml)	Range (RU/ml)
<1	8	105	43-324
2-4	27	80	36-197
5-11	73	69	35-132
12-15	61	77	36-138
16 - ≥19	49	61	27-107
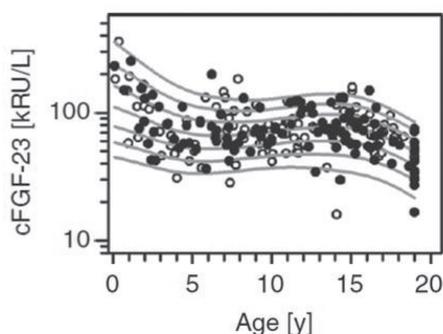
Reference Fischer DC, Mischek A, Wolf A, Rahn A, Salweski B, Kundt G and Haffner D.
Pediatric reference values for the C-terminal fragment of fibroblast-growth factor-23, sclerostin, bone-specific alkaline phosphatase, and isoform 5b of tartrate resistant acid phosphatase. Annals of Clinical Biochemistry 2012; 49: 546 -553.

Species Human, Cynomolgus Macaque

FGF-23 (C-Term) Mouse

FIBROBLAST GROWTH FACTOR 23

C-terminal	
Cat. No.	60 - 6300
Tests	96
Method	ELISA
Range	30 – 1000 pg/ml (can be extended to 3000 pg/ml)
Sensitivity	4 pg/ml
Incubation time	3.5 hours
Sample volume	25 µl
Sample type	Serum, plasma, cell culture
Sample preparation	FGF-23 molecule is very unstable. Therefore samples should be assayed immediately or stored frozen at -20°C or below. Avoid repeated freeze/thaw cycles.
Species	Mouse, rat, canine, porcine

FGF-23 Intact, Human (Kainos)

FIBROBLAST GROWTH FACTOR 23

C-terminal	
Cat. No.	CY-4000
Tests	96
Method	ELISA
Range	8 – 800 pg/ml
Sensitivity	3.0 pg/ml
Incubation time	3.5 hours
Sample volume	50 µl
Sample type	Serum / Heparin- and EDTA Plasma
Sample preparation	It is recommended to collect the sample in the morning after a 12-hour fasting period. Intact FGF-23 is very instable. Therefore, collection and testing or storage should take place promptly. Store samples at -20 °C or below. Avoid repeated freezing and thawing of specimens.
Reference values	10 – 50 pg/ml
Species	Human, Rat, Mouse

Klotho, Human

Cat. No.	27998
Tests	96
Method	ELISA
Range	93.75 - 6000 pg/mL
Sensitivity	6.15 pg/mL
Incubation time	2 hours
Sample volume	100 μL
Sample type	Serum, EDTA plasma
Sample preparation	Collection, testing and samples storage should take place promptly. Store samples at -20 ℃ or below. Avoid repeated freezing and thawing of specimens.
Reference values	239 – 1266 pg/mL
Species	Human
Specificity	No cross-reactions observed with: osteopontin, human VEGF or PDGF.
Intended use	Klotho is a single-pass trans-membrane anti-aging protein (1014 amino-acids, 130 kDa, chromosomic location in 13q12 in humans) that has been recently shown to have wide and important biological effects. Its expression occurs mainly in the kidney and in the parathyroid. Its extra-cellular domain is wide whereas its intra-cellular part is very short; it also exists as a soluble protein.
	In humans, Klotho polymorphisms have been associated both in a positive and negative way to bone mineral density, life expectancy, cardiovascular events (e.g., ischemic stroke, carotid atherosclerosis), biomarkers of metabolic syndrome (e.g., uric acid levels, lipid and glucose metabolisms), and even to cognitive ability.

NOTES

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