Implications of the Phosphate Regulatory FGF-23/Klotho System in Cardiovascular Disease

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1 Introduction

Alterations in phosphorus homeostasis are involved in multiple physiopathologic mechanisms and clinical processes. These alterations are very frequent in chronic kidney disease (CKD) patients, in whom phosphorous retention and hyperphosphatemia are common features that contribute to explain the high cardiovascular morbidity and mortality in this population.

However, growing evidence in recent years demonstrated that not only variations in phosphate levels, but also in phosphate-regulatory factors are linked with morbidity and mortality (Kuro-o, 2010a; Block et al., 2004; Foley, 2009; Kestenbaum et al., 2005; Tonelli et al., 2005a; Parker et al., 2010; Mirza et al., 2009a; Levin et al., 2007). This suggests that both elements could be related to cardiovascular adverse risk factors and outcomes, including atherosclerosis, arterial calcification, vascular stiffness, and left ventricular hypertrophy (LVH) (Block et al., 2004; Shuto et al., 2009; Zisman et al., 2010).

Classically, regulation of serum calcium and phosphate levels was believed to be achieved by a feedback between two main endocrine factors, calcitriol or 1,25-dihydroxyvitamin D3 (the active form of vitamin D) and parathyroid hormone (PTH) (Foley, 1998a; Foley, 1998b). However, in addition to PTH and calcitriol, recent studies have identified a novel regulator of phosphate levels: the fibroblast growth factor (FGF) 23 (Block et al., 2004; Shimada et al., 2001) which is actually considered the as the principal regulator of phosphatemia inducing phosphaturia and inhibiting calcitriol synthesis in the kidney, therefore maintaining systemic phosphate homeostasis. Like other members of the endocrine FGFs group, FGF-23 requires a co-factor for bind and activate its cognate receptors (Itoh, 2010; Kuro-o, 2008). To exert its actions, FGF-23 requires Klotho, a single-pass transmembrane protein that is predominantly expressed in the kidney (Kurosu et al., 2006; Yu et al., 2005).

The appearing of this novel phosphatemic central regulator not only has changed our understanding about mineral metabolism regulation, but has also provided novel implications in the relationship between the phosphate imbalance and the increased cardiovascular risk. Thereby, diverse studies have showed the potential role of FGF-23 excess in cardiovascular disease (CVD) incidence in patients with and without kidney impairment, including increased mortality risk (Parker et al., 2010; Gutiérrez et al., 2008; Jean et al., 2009), LVH (Mirza et al., 2009b; Gutiérrez et al., 2009), vascular dysfunction (Mirza et al., 2009c), atherosclerosis (Mirza et al., 2009c), and cardiovascular events (Kanbay et al., 2010). Moreover, diverse studies have confirmed the role of Klotho in not FGF-23-related favourable effects over the vascular system, including calcitriol and nitric oxide synthesis, suppression of Wnt signalling, oxidative stress, and vascular calcifications (Kuro-o, 2009a). The recently described expression of Klotho in human vascular tissue and vascular smooth muscle cells (VSMCs) (Donate-Correa et al., 2013; Lim et al., 2012) may partially explain these effects of the FGF-23/Klotho axis over the cardiovascular system. Moreover, a soluble form of Klotho can be also detected in blood, urine, and cerebrospinal fluid, which appears to have diverse endocrine actions.

Currently, the potential utility of the mineral metabolism regulators, especially FGF-23 and Klotho, as clinical biomarkers is an area of intense investigation, especially focused in renal patients. However, there is a long way to go in which FGF-23 and Klotho will have to prove its usefulness in the early diagnosis and its prognostic value in CVD.
2 FGF-23/KLOTHO System: A Master Regulator of Phosphate Metabolism

The classic view of the regulation of serum calcium and phosphate levels is product of a feedback between calcitriol and PTH, by counterbalanced intestinal uptake, mobilization from bone, and renal excretion (Berndt & Kumar, 2003; Dusso et al., 2005). Calcitriol is synthesized in the kidney and acts in the gut increasing the absorption of dietary calcium and phosphate, and in the bone, promoting mobilization of these ions (Figure 1). As result, blood levels of both phosphate and calcium trend to increase. PTH is secreted from parathyroid glands in response to hypocalcemia, promoting bone resorption and stimulating calcitriol synthesis in the kidney. In addition, PTH induces phosphaturia by diminishing the reabsorption of phosphate in the kidney. Therefore, the final effect of PTH is an elevation of calcium without increase of phosphate blood levels (Berndt & Kumar, 2007).

![Figure 1](image-url): The physiological regulation of phosphorus serum levels is based on the interaction between vitamin D, PTH and FGF-23.

Thereby, variations in components of phosphate regulatory system traditionally associated with increased cardiovascular risk have been low calcitriol and high PTH levels (Foley, 1998a; Foley, 1998b). However, in addition to PTH and calcitriol, recent studies have identified a novel regulator of phosphate levels: FGF-23 (Block et al., 2004; Shimada et al., 2001) which is actually considered the as the principal regulator of phosphatemia inducing phosphaturia and inhibiting calcitriol synthesis in the kidney, therefore maintaining systemic phosphate homeostasis. FGF-23 was first identified as the primary cause of two diseases characterized by phosphate-wasting syndromes originated by impaired renal phosphate re-absorption and low levels of calcitriol (Fukumoto et al., 2007): autosomal dominant hypophosphatemic rickets (ADHR) (Consortium ADHR, 2000) and tumor-induced osteomalacia (TIO) (Shimada et al., 2001), respectively. In contrast, low level of FGF-23 was identified as responsible of phosphate-retaining...
symptoms observed in patients with familial tumoral calcinosis (FTC) (Garringer et al., 2006). These observations allowed the identification of FGF-23 as a critical factor in the physiological regulation of phosphate.

FGF-23, together with FGF-19 and FGF-21, belongs to the particular group of endocrine FGFs (It-oh, 2010), which are characterized by presenting low affinity for their FGF receptors (FGFRs), and the obligate requirement of a cofactor to bind and activate these receptors (Schlessinger et al., 2000). Klotho protein is the cofactor implicated in the binding and activation of FGFRs by FGF-23. This explains why hyperphosphatemic phenotypes of Klotho-deficient mice are replicated in FGF-23-deficient mice (Razzaque & Lanske, 2006; Shimada et al., 2004a). Organ-specific expression of Klotho restricts the activity of FGF-23 to few tissues including parathyroid glands, choroid plexus, renal distal tubules, and recently vascular tissue (Kuro-o et al., 1997; Kuro-o, 2009a; Donate-Correa et al., 2013; Lim et al., 2012; Li et al., 2004).

The Klotho gene was identified in a serendipitous experiment as a mutated gene in a mice strain that inherits a premature-ageing phenotypes including atherosclerosis, endothelial dysfunction, vascular calcification, hyperphosphatemia and shortened life span (Kuro-o et al., 1997). The first clue to identify the putative cofactor required by FGF-23 for binding FGFRs came from verifying that the phenotypes of Klotho-deficient mice were replicated in FGF-23-deficient mice (Razzaque & Lanske, 2006), which not only were hyperphosphatemic, but also shared all the premature-ageing symptoms of Klotho-null mice (Shimada et al., 2004a). This allowed to hypothesize a coordinated action of both factors to transmit the same signal pathway, explaining why mice lacking Klotho, FGF-23, or both exhibit identical phenotypes, as well as the insensitivity of Klotho null mice to the high levels of FGF-23 present in this mutant (Nakatani et al., 2009).

FGF-23 is secreted by osteocytes and osteoblasts in humans and mice in response to dietary phosphate intake to maintain phosphorus homeostasis (Kuro-o, 2009b; Antoniucci et al., 2006). It acts increasing the renal phosphate excretion and inhibiting the calcitriol synthesis by reducing the traffic and/or expression of the sodium-phosphate cotransporters type 2 (Na/Pi-2a and Na/Pi-2c), responsible of phosphate reabsorption (Miyamoto et al., 2004; Segawa et al., 2007; Segawa et al. 2003; Shimada et al., 2004b; Kuro-o, 2010b), and decreasing renal activation of calcitriol, leading to a reduction in the intestinal absorption of phosphate (Liu et al., 2006; Wang & Sun, 2009) (Figure 1).

GF-23 also inhibits the expression and production of PTH in parathyroid glands (Ben-Dov et al., 2007; Krajisnik et al., 2007). This results in lower PTH and calcitriol levels (since PTH also reduces renal calcitriol synthesis) and subsequently in a lesser phosphate absorption from gut and resorption from bone. The regulatory feedback loops among kidney, bone, and parathyroid glands is closed by the stimulatory effects exerted by calcitriol and PTH over FGF-23 expression and/or secretion in bone (Kuro-o, 2009a; Shimada et al., 2004b; Saji et al., 2009; Perwad et al., 2005; López et al., 2011) (Figure 1).

3 Emerging Role of Phosphorus as Predictor of Cardiorenal Progression Disease

Inorganic phosphate plays essential roles in every biological process since is an integral part of important compounds including nucleic acids, cyclic nucleotides, phospholipids, and the energy metabolism intermediates (Gaasbeek & Meinders, 2005). Although in humans almost all the phosphorous is found in bone and teeth, and only a low percentage exists as serum phosphate (Razzaque, 2009), the regulation of this
percent is extremely important. This regulation is achieved balancing dietary absorption, bone formation, and renal excretion, as well as by equilibration with intracellular stores.

Disturbances in phosphorous homeostasis are involved in multiple physiopathologic mechanisms and clinical processes. Hypophosphatemia causes muscle weakness and circulatory collapse, whilst hyperphosphatemia is associated with endothelial apoptosis, vascular calcification and LVH, and constitutes a risk factor for cardiovascular mortality (Di Marco \textit{et al}., 2008; Jono \textit{et al}., 2000; Reynolds \textit{et al}., 2004; Ellam & Chico, 2012; Ayus \textit{et al}., 2005). Importantly, beyond those extreme pathological states, recent observational data have linked narrow variations in phosphate levels and, as will be discussed later, in phosphate-regulatory factors, with cardiovascular events and mortality (Kuro-o, 2010a; Block \textit{et al}., 2004; Foley, 2009; Kestenbaum \textit{et al}., 2005; Tonelli \textit{et al}., 2005a; Parker \textit{et al}., 2010; Mirza \textit{et al}., 2009a,b,c; Adeney \textit{et al}., 2009), suggesting a very intriguing link between phosphate and cardiovascular disease (Kuro-o, 2010a; Block \textit{et al}., 2004; Shuto \textit{et al}., 2009).

Although most of studies linking phosphate with cardiovascular risk and mortality have been carried out in CKD patients, in which phosphate overload and overt hyperphosphatemia are usual features (Palmer \textit{et al}., 2011; Tentori \textit{et al}., 2008; Block \textit{et al}., 2004), increased serum phosphate within the normal range has also been associated with adverse clinical outcomes in individuals free of CKD and CVD in the community (Dhingra \textit{et al}., 2007), in subjects with prior acute myocardial infarction and without impaired kidney function (Tonelli \textit{et al}., 2005b), and in community-dwelling adults after adjusted for glomerular filtration rate (GFR) (Foley \textit{et al}., 2008).

The pathophysiological basis of the increased cardiovascular risk linked to phosphorous is explained by subyacent vascular calcification (Shanahan \textit{et al}., 2011), atherosclerosis (Ellam \textit{et al}., 2011), endothelial dysfunction (Shuto \textit{et al}., 2009), and LVH (Ayus \textit{et al}., 2005).

In CKD patients, in whom hyperphosphatemia, low levels of calcitriol and secondary hyperparathyroidism (SHPT) are common features, FGF-23 serum levels are increased in early stages of CKD, even before serum phosphate, PTH concentrations or vitamin D levels had become abnormal (Isakova \textit{et al}., 2011; Gutiérrez \textit{et al}., 2005) (Figure 2).

![Figure 2: Variations in FGF-23, Klotho, PTH, Vitamin D, and phosphate levels during the progression of CKD. The first alteration is the decrease in Klotho expression, which provokes an increase in FGF-23 levels. FGF-23 lowers circulating vitamin D production in kidney which increases PTH expression. The resultant is an hyperphosphatemic, low calcitriol and high PTH state in late stages of CKD.](image-url)
Diverse studies point to a progressive increase in FGF-23 as the renal function declines (Gutiérrez et al., 2005; Larsson et al., 2003). In dialysis patients, the raise can be up to 1000-fold higher than in healthy individuals (Larsson et al., 2003). This elevation may reflect a compensatory response to maintain normal phosphatemia, which tend to increase when glomerular filtration rate (GFR) declines. An alternative hypothesis suggests that the increase of FGF-23 reflects an end-organ resistance to the phosphaturic stimulus of FGF-23 because of a deficiency in Klotho (Koh et al., 2001). Similarly, it is also unknown if the raise in FGF-23 represents an increased secretion from bone, a decreased degradation, or both (Wolf, 2009).

4 Role of FGF-23 in Cardiorenal Disease as Biomarker. Findings from in vitro/vivo Studies and Observational Studies

Diverse association studies have revealed that FGF-23 is an independent predictor of survival and cardiovascular morbidity both in populations with (Gutiérrez et al., 2008; Kanbay et al., 2010; Isakova et al., 2011) and without (Parker et al., 2010; Mirza, Larsson et al., 2009b; Mirza et al., 2009c) kidney function impairment. It is noteworthy the stronger magnitude of the association between mortality and CVD with FGF-23 than that observed with serum phosphate. This has been reflected in several studies including the Heart and Soul Study, that comprises a cohort of heart disease patients with normal-kidney function (Parker et al., 2010) which is not attenuated in early CKD patients when adjusting for serum phosphate (Gutiérrez et al., 2008; Kanbay et al., 2010; Isakova et al., 2011).

These results have led to consider whether FGF-23 acts simply as a phosphate disarrangement biomarker or might directly targets the cardiovascular system. If this is true, it is plausible to think in FGF-23-mediated toxic actions in different organs, including the cardiovascular system.

Several clinical studies have determined the association between FGF-23 and the incidence of specific cardiovascular injuries and risk factors. A direct correlation between FGF-23 level and the presence of LVH has been reported in patients with advanced CKD (Gutiérrez et al., 2009; Faul et al., 2011) and in elderly population (Mirza et al., 2009a). Likewise, other studies in CKD patients have reported the association between FGF-23 and vascular dysfunction (Mirza et al., 2009b), atherosclerosis (Mirza et al., 2009c), coronary artery calcifications (Morena et al., 2012), and increased risk of mortality and cardiovascular events (Kanbay et al., 2010; Kendrick et al, 2011) (Table 1).

A mechanism suggested to explain these associations by direct effects of FGF-23 is a Klotho-independent low-affinity binding to FGFRs, which would occur under conditions characterized by elevated FGF-23 concentrations, such as CKD (Yu et al., 2005; Larsson et al., 2003; Wang et al., 2008). This possibility has been recently confirmed in experimental studies, where intravenous and intramyocardial administration of FGF-23 resulted in hypertrophy of rat cardiomyocytes and LVH, demonstrating the role of FGF-23 in the pathogenesis of LVH (Faul et al., 2011). These findings strongly suggests that a component of cardiovascular risk in CKD patients could be directly attributable to FGF-23 (Faul et al., 2011), although this must be confirmed in further studies.
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<th>Variable</th>
<th>Study Population</th>
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<td>Higher levels of FGF-23</td>
<td>General Population</td>
<td>Vascular dysfunction</td>
<td>Mirza et al., 2009b</td>
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<td></td>
<td>General Population</td>
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<td>Hemodialysis adult patients</td>
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<td>Hemodialysis patients</td>
<td>Increased risk of mortality and vascular calcifications</td>
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<td>Patients with stable CAD</td>
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<td>Klotho gene polymorphisms</td>
<td>General Population</td>
<td>Increased risk of coronary artery disease</td>
<td>Arking et al., 2003; Imamura et al., 2006; Jo et al., 2009; Rhee et al., 2006</td>
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Table 1: FGF-23 and KLOTHO human cardiovascular events related studies. CKD: Chronic kidney disease; LVH: left ventricular hypertrophy; CAD: coronary artery disease.

Concerning vascular damage, only a few studies have been designed to investigate the effects of FGF-23 on vessel integrity. The recent demonstration of the expression of FGFRs and Klotho in the human vascular wall (Donate-Correa et al., 2013; Lim et al., 2012), together with experimental studies showing that binding of FGF-23 to vascular wall FGFRs could, theoretically, generates adverse effects (Wolf, 2010), reinforce the possibility of a direct FGF-23 effect upon vascular wall.

Independently of the promiscuity of binding of FGF-23, effects on vascular tissue might include regulation of Na/P cotransporters PiT-1 and/or PiT-2 in a similar way to the inhibitory effect exerted over Na-Pi2a and 2c cotransporters in kidney. Even more, inhibitory effect on 1-alpha-hydroxylase, expressed by endothelial and VSMCs, might result in reduced local synthesis of vascular calcitriol, a hormone with a protective role against vascular diseases (Judd & Tangpricha, 2009).

Although additional research is needed to confirm the existence of this new action model on the vascular tissue and to examine its substantial clinical implications, strategies aimed to decrease elevated FGF-23 levels in CKD could emerge as a novel renal disease therapeutic approach. Proposed strategies would include phosphorus restriction (Saito et al., 2000), administration of phosphate binders (Oliveira et al., 2010; Koiwa et al, 2005), and even the use of specific antibodies directed against FGF-23 (Wolf, 2010).
Figure 3: Under physiological conditions, the endocrine regulation of phosphate metabolism takes place by three different feedbacks: circulating vitamin D activates the FGF-23 promoter in bone. The secreted FGF-23 inactivates the production of vitamin D in kidney. On the contrary, PTH activates production of vitamin D. Finally, FGF-23 shuts off the PTH promoter in parathyroid glands. Pathologic processes derived from early-stage CKD are related with the increased secretion of FGF-23 from bone acting in the kidney, were Klotho is under-expressed, to maintain a neutral phosphate balance. This results in suppression of renal vitamin D production that triggers the early development of secondary hyperparathyroidism. The excess of FGF-23 is also associated to vascular dysfunctions, atherosclerosis and left ventricular hypertrophy (LVH).

5 Role of Circulating Klotho in Cardiorenal Disease as Biomarker and Therapeutic Factor

Research on Klotho has opened an extraordinary field because its implications in a multitude of biological processes, many of them related to human longevity. Klotho human gene comprises 5 exons and encodes a single-pass transmembrane protein of 1012 amino acids with a large amino-terminal extracellular domain, consisting in two internal repeat sequences (KL1 and KL2), and two short membrane-spanning (21 amino acids) and intracellular carboxyl (11 amino acids) domains.

Soluble Klotho predominates in humans over the membrane form, declines with age (Xiao et al., 2004) and can be generated through two different pathways: by an alternative Klotho mRNA splicing putatively encoding only the KL1 (Matsumura et al., 1998) and by a proteolytic cleavage by membrane-anchored A Desintegrin and Metalloproteinases (ADAM)-17 and ADAM-10, that release the full-length extracellular domain into the extracellular space (Figure 4). This last processing could be important in the vessels since the gene expression level of ADAM-17 has been directly correlated with Klotho gene expression in human aortic thoracic samples (Donate-Correa et al., 2013). Furthermore, the aminoacidic
sequence between the repeats KL1 and KL2 (Lys-Lys-Arg-Lys) forms a potential site for proteolytic cleavage. Although these fragments are not detected in human serum and cerebrospinal fluid, the existence of the alternatively spliced KL1 transcript in urine should not be excluded and it would be interesting to determine whether these fragments have some biological properties.

Figure 4: Representation of the complex formed by FGF-23, Klotho and the FGFRs in the cellular membrane. Alternative splicing of the mRNA generating the membrane and the soluble form (KL1) of Klotho and proteolytic cleavage by membrane proteinases that release the full-length extracellular domain into the extracellular space.

Although most part of the work with Klotho has been focused in its role as renal cofactor for the binding of FGF-23, the existence of a soluble form of Klotho and the expression of Klotho in the vascular tissue, allows consider this molecule as a novel circulating factor able to exert direct effects in multiple organs, including cardiovascular system. Klotho deficient-mice model shows a human-like aging syndrome that includes accelerated arteriosclerosis associated with extensive medial calcification of the aorta, and both medial calcification and intimal thickening of medium-sized muscular arteries (Saito et al., 2000). In addition, they exhibit impaired angiogenesis (Fukino et al., 2002) and endothelial dysfunction (Saito et al., 1998; Shiraki-Iida et al., 2000) which can be ameliorated by in vivo gene delivery of the Klotho gene, by parabiosis with the Klotho wild type specimen (Saito et al., 2000) or by administration of
soluble Klotho which revert many age-related disorders (Chen et al., 2013). Klotho deficient mice were also associated with higher phosphorous levels and severe calcification (Hu et al., 2011).

Recent experimental studies confirm soluble Klotho protective effects upon vascular system. These effects include a role in maintaining endothelial wall homeostasis and in promoting the vascular health (Saito et al., 2000; Saito et al., 1998; Nagai et al., 2000) triggering its absence endothelial dysfunction and vascular calcification in experimental models (Hu et al., 2011; Nagai et al. 2000).

In addition, genetic variation studies have demonstrated that Klotho gene polymorphisms might be associated with longevity (Arking et al., 2002) and coronary artery disease (CAD) (Arking et al., 2003; Imamura et al., 2006; Jo et al., 2009; Rhee et al., 2006) (Table 1). Although at least one of these variations influence to Klotho gene expression (Kawano et al., 2002), the impact of serum levels of this protein on human coronary arteries remains to be clearly elucidated. Only one reported a decrease in the incidence of CAD with increasing tertile of plasma Klotho in older community-dwelling adults although this difference did not reach statistical significance (Semba et al., 2011). CAD is mainly caused by established coronary arteriosclerosis derived from endothelial dysfunction which could be developed by low Klotho levels. Therefore, it has become evident the necessity to evaluate the contribution of endothelial protector serum Klotho to CAD risk.

Many vascular actions are proposed to explain Soluble Klotho vascular-protective activities. Endothelial protection could be due to a Klotho-mediated up-regulation of nitric oxide (NO) production in endothelial cells. This hormone besides acting as vasodilatador, prevents atherogenesis by suppressing VSMCs proliferation and by inhibiting adhesion molecules expression and platelet aggregation (Quyyumi et al., 1998). Klotho gene deficiency reduces the capability of vasodilatation in aorta and arterioles of mice and attenuates the excretion of urinary nitric oxid metabolites (Saito et al., 2000). This deficiency is reverted in parabiosis with wild-type mice and also by adenovirus-mediated Klotho gene delivery in the atherogenic model Otsuka Long-Evans Tokushima Fatty (OLETF) (Saito et al., 1998), preventing adverse vascular remodelling.

Moreover, Klotho is also involved in the modulation of inflammation. In human umbilical vein endothelial cells (HUVECs), addition of recombinant Klotho suppresses TNFα induced expression in the endothelium of adhesion molecules involved in the pathogenesis of vascular disease (Maekawa et al., 2009).

On the other hand, it has been reported that soluble Klotho protein is able to binding to various Wnt family members, and that Wnt–Klotho interaction results in the suppression of Wnt biological activity (Liu et al., 2007; Zhou et al., 2013). This signal is essential for the proliferation and survival of stem cells, but continuous Wnt exposure may contribute to exhaustion and depletion, as well as accelerated cellular senescence (Kirstetter et al., 2006). Klotho-mediated attenuation of Wnt signalling could contribute to the anti-ageing properties of Klotho by avoiding the continuous activation of Wnt signalling and the senescence of stem cells (Kuro-o, 2010b) and eliciting renoprotective effects (Zhou et al., 2013).

Experimental studies show that calcitriol administration promotes expression of Klotho via the activation of vitamin D receptor (Tsujikawa et al., 2003; Forster et al., 2011). A recent work has demonstrated that administration of alfacalcidol, a vitamin D receptor activator, promoted an up regulation of Klotho gene expression in the kidney of nephrectomized spontaneously hypertensive rats (Fukui et al., 2011). According this, Klotho variants associated with lower Klotho gene expression have been associated with a decrease in survival of dialysis patients, more pronounced among patients not treated with active forms of vitamin D (Friedman et al., 2009).
Additionally, Klotho is also able to inhibit vascular calcification. As FGF-23 does in Kidney, soluble Klotho inhibits Na/Pi-2a and Na/Pi-2c, and also the Na/Pi cotransporters type 3 (Na/Pi-3 also known as Pit 1 and Pit 2) (Hu et al., 2011; Hu et al., 2010). These last two are widely expressed in tissues such as intestinal epithelium, liver, lung, heart and smooth muscle cells (Jono et al., 2000; Kakita et al., 2004; Okuda et al., 2006). The addition of recombinant soluble Klotho protein to VSMCs cultures is able to decrease high Pi-induced calcification by diminishing expression of Na/Pi-3 cotransporters (Hu et al., 2011). Secreted renal and/or vascular soluble Klotho might protect against vascular calcification through this inhibition of Na/Pi-3 expression.

Since soluble Klotho decreases at early CKD stages (Pavik et al., 2013; Shimamura et al., 2012), the utility of soluble Klotho as a biomarker CKD progression has been suggested. Diverse observational studies that relate low circulating or urinary klotho levels with adverse kidney disease outcome (Kim et al., 2013; Kitagawa et al., 2013; Akimoto et al., 2012; Satoh et al., 2012). More data from larger prospective longitudinal studies are required to validate this possibility. Similarly, some studies suggest that administration of Klotho may have therapeutic possibilities in treating renal disease (Mitani et al., 2002).

6 Utility of FGF-23 and Klotho as Biomarkers in the Clinical Practice

The recent discovery of the FGF-23/Klotho system has provided potentially reliable biomarkers with clinical utility especially in patients with kidney disease. But their potential utility seems to transcend the field of renal failure, and cover areas such as cardiovascular disease. However, several important obstacles must be overcome in order to include these molecules as reliable biomarkers in clinical practice.

Although several assay kits measure circulating FGF-23 in the intact form (iFGF-23) alone or both intact and the carboxi-terminal fragment (cFGF-23) product of degradation, it is unclear if all the assays provide comparable sensitivity for patients with different stages of renal function. Some studies suggest that measurements of iFGF-23 rather than cFGF-23 may be more physiologically relevant, whereas other works show significant associations only with cFGF-23. However, a recent clinical study has demonstrated that virtually all detectable FGF-23 is in the active form, and thus, measurements obtained with iFGF-23 and cFGF-23 assays would reflect the same circulating moiety (Shimada et al., 2010). Additional studies are needed to determine whether this occurs regardless of kidney function and if this value can be a marker of cardiovascular risk in the renal patient.

The potential use of this protein as a biomarker has originated a great interest in developing automated methods to improve the performance of the FGF-23 immunoassays overcoming the limitations of the ELISA kits. A new automated chemiluminiscence immunoassay has been recently developed to measure intact and cFGF-23 concentrations (Shimizu et al., 2012). However, current assays should be evaluated and standardized more rigorously and further studies are also needed to establish clearly the influence of preanalytical factors. Moreover, population-based studies focused on defining clear cutoff values for clinical risk assessment are scarce. Furthermore, some are referred to specific assays and overlook variables which could alter the measures, so the utility is limited. Before its introduction in the clinical practice, we should get more robust reference values and establish and validate the recommended cutoff for treatment targets.
In contrast to FGF-23, studies that explore the relationships between human sKlotho levels and diverse cardiovascular clinical phenotypes are scarce due to the lack, until recently, of a reliable assay for measuring this protein (Yamazaki et al., 2010). Paradoxically, although Klotho protein is expressed mainly in the kidney, renal investigators only have fixed their attention on this protein in recent years. Some of these works have been focused on the potential utilization of Klotho as a biomarker in CKD with prognostic and diagnostic capabilities.

Soluble Klotho is subjected to diurnal variations decreasing to their circadian nadir at midnight and a maximum in the early morning (Carpenter et al., 2010). Although the physiological significance of this pattern is unknown, it should be taken into account for drawing blood and urine samples. Similarly, it has also been reported a negative relationships between Klotho levels and age in healthy volunteers (Yamazaki et al., 2010) in patients with X-linked hypophosphataemia (Carpenter et al., 2010), and in children with CKD (Wan et al., 2013). There were no apparent differences regarding gender. However, much further research is needed to validate and standardize this biomarker in the translational arena, including studies to define reference values and measure its biological variability.

Currently, the human database for Klotho is scarce and is expected that the number of human studies will be increased in the very near future. Larger cohorts are needed and, more importantly, validate in different cardiovascular and renal injuries stages the ELISA kit currently employed by most of the studies.

7 Conclusion and Future Prospects

Nowadays, the major interest to the clinical application of FGF-23/Klotho research is related to CKD. Increased phosphate levels associated to reduced renal function in these patients has been related to progression of cardiovascular complications and enhanced morbimortality. However, serum phosphate level remains normal until advanced reduction in renal function, and in addition, the magnitude of its association with deleterious effects within the physiological range is very small (Kestenbaum et al., 2005; Tonelli et al., 2005a; Dhingra et al., 2007) unlike increases in FGF-23 and decreases in Klotho, which have been linked with cardiovascular morbidity and mortality independently of phosphatemia (Kuro-o, 2010b; Block et al., 2004; Kestenbaum et al., 2005; Tonelli et al., 2005a; Gaasbeek et al., 2005; Razzaque, 2009). Importantly, these association remains in population without impaired kidney function.

Further studies are necessary to clarify the regulatory mechanisms controlling FGF-23 and Klotho expression and to evaluate the potential translation to the clinical setting. Another potential therapeutic option could be directed to elevation of Klotho levels. Although results depicted in this chapter clearly point to Klotho as a potential therapeutic agent in mineral-cardiovascular disorders, further studies are needed to evaluate the reliability and practical utility of this protein. In example, relationships between circulating Klotho and many clinical phenotypes in which this protein could be involved has not been extensively studied because of the scarce clinical studies measuring blood soluble Klotho in humans.

Finally, many questions about the direct effect of FGF-23 on CVD have emerged. For example, it is not clear if human hereditary hypophosphatemic rickets, caused by high FGF-23 systemic levels, is accompanied by increased atherosclerosis (Ellam et al., 2012). Similarly, overexpression of FGF-23 in mice is not associated with adverse cardiovascular effects when hypophosphatemia is corrected through
changes in diet (Ellam et al., 2012; Liu et al., 2009). However, a possible explanation for this is the reported absence (Lim et al., 2012), unlike humans, of Klotho expression in rat VSMCs.

Another open question is the elucidation of the contribution of soluble vascular Klotho in advanced stages of CKD, where low serum Klotho levels are frequent. It is unknown if vascular expression is also lowered in this states or remains physiologic levels. Anyway, it is probably that vascular derived Klotho plays a more important role in these patients since renal contribution is dramatically diminished.

FGF-23 and Klotho are emerging as unprecedented biomarkers for, but no exclusively, nephrologists. However, there is a long way to go in which FGF-23 and Klotho will have to prove its usefulness in the early diagnosis and its prognostic value.

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