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On the relationship between proteinuria and plasma phosphate

de Seigneux Sophie[a], Wilhelm-Bals Alexandra[b], Courbebaisse Marie[c]

[a] Service and laboratory of Nephrology, Department of Internal Medicine Specialties and Department of Physiology and Metabolism, University Hospital and University of Geneva, Switzerland

[b] Unité Pédiatrique Néphrologique Romande, Department of Paediatrics, University Hospital of Geneva and University Hospital Centre of Lausanne, Switzerland

[c] Service of Functional Explorations, Department of Physiology, Hôpital Européen Georges Pompidou, Paris, France

Summary

Albuminuria is strongly associated with renal and cardiovascular outcomes independently of renal function level. However, the pathophysiology of these associations is debated. In chronic kidney disease (CKD), phosphate retention participates in cardiovascular events and increased cardiovascular mortality. We hypothesised that albuminuria may modulate tubular phosphate handling by the kidney. To verify this hypothesis, we first studied the association between phosphataemia and albuminuria in children with nephrotic syndrome and in adults with CKD. In both cases, higher albuminuria was associated with higher phosphate level, independently of glomerular filtration rate. We further tried to decipher the molecular mechanisms of these observations. Using animal models of nephrotic proteinuria, we could show that albuminuric rats and mice had abnormally elevated sodium-phosphate apical co-transporter expression, despite elevated fibroblast growth factor 23 (FGF23). The FGF23 downstream pathway was inhibited despite elevated FGF23 levels. Klotho protein expression was also lower in proteinuric animals compared to controls. Finally, albumin had no direct effects on phosphate transport in cells. Altogether, we show that albuminuria induces alteration of phosphate tubular handling, independently of glomerular filtration rate. The mechanisms involved appear to include Klotho down-regulation and resistance to FGF23. This observation may link albuminuria to increased cardiovascular disease via altered phosphate handling. Finally, this observation opens up further opportunities to better understand the link between albuminuria, Klotho, FGF23 and phosphate handling.

Key words: *albuminuria, phosphate, chronic kidney disease, FGF23, Klotho, cardiovascular*

Correspondence:
Sophie de Seigneux, Service et laboratoire de Néphrologie, 4 rue Gabrielle Perret gentil, CH-1205 Genève, Sophie.de-seigneux[at]hcuge.ch

The predictive value of proteinuria/albuminuria in chronic kidney disease (CKD)

Healthy urine is devoid of significant amount of albumin, and contains only low levels of some physiologically secreted proteins, such as uromodulin [1]. For several years, the presence of excessive protein in the urine (mainly albumin) has been linked to the prognosis of renal function, in both at-risk patients and in the general population [2–5]. In addition to renal complications, albuminuria is also strongly predictive of cardiovascular disease and mortality [2, 3, 6, 7], independently of glomerular filtration rate (GFR). This has led to the inclusion of albuminuria in addition to estimated GFR in the definition of chronic kidney disease (CKD) [8, 9].

The strong predictive value of albuminuria for CKD progression is not contested [2–5]. However, the causal role of albuminuria in CKD progression is not unanimously recognized among nephrologists. Indeed, the belief that albuminuria could be only a marker of glomerular injury, leading to fibrosis, is still quite popular. However, several lines of evidence clearly demonstrate that albumin or other filtered proteins have pro-inflammatory, pro-apoptotic and pro-fibrotic effects on both proximal and distal tubular cells [10–12]. Whereas absorption of albumin is mediated by the albumin/cubulin system in the proximal tubule [1, 13, 14], the route of absorption of albumin in the distal nephron is unclear and the 24p3R could play a role [15, 16]. The detrimental role of albumin on tubular cells may secondarily induce tubulointerstitial fibrosis. Indeed, several recent diverse observations convincingly show that injury to tubular cells alone, in the absence of glomerular lesions, may lead to kidney interstitial fibrosis, and likely not through epithelial to mesenchymal transition [17, 18].

How albuminuria and proteinuria are related to cardiovascular risk is even more uncertain. Although this strong relationship suggests that albuminuria should be considered as a cardiovascular risk marker, its causative role in the pathophysiology of cardiovascular lesions is undetermined. Importantly, decreasing albuminuria by either ACE inhibitors or more recently, via SGLT2 inhibitors, reduces both renal and cardiovascular risks [19, 20]. Albuminuria may be the signature of endothelial injury, and therefore indicate diffuse endothelial lesions associated with cardiovascular disease [21, 22]. In contrast, albuminuria may play a direct pathophysiological role, directly increasing cardiovascular disease risk.

Phosphate retention and cardiovascular mortality in kidney disease

CKD is associated with a major increase in cardiovascular events and mortality. This association is explained by a high prevalence of traditional risks factors in CKD patients, such as diabetes and hypertension for example. However, some non-traditional cardiovascular risk factors are specific to CKD patients. Among those, abnormal phosphate handling related to decreased renal filtration may play an important role.

Phosphate is absorbed by the gut. The main sources of phosphate in the diet include protein-rich and pr-processed foods due to the presence of preservatives. Phosphate is mostly passively absorbed in the intestine. The bones are the major reservoir for phosphate in the body. Excess phosphate ingested is excreted in the urine to maintain a neutral daily phosphate balance [23, 24].

Most of the phosphate is filtered at the glomerulus. More than 85% is reabsorbed in the proximal tubule by sodium/phosphate co-transporters (mainly NPT2a and NPT2c in humans). Major hormonal regulators of tubular phosphate reabsorption include parathormone (PTH) and fibroblast growth factor 23 (FGF23). PTH increases phosphaturia by inducing endocytosis and degradation of sodium/phosphate co-transporters in the proximal tubule. PTH is mainly secreted in response to changes in calcaemia, and to a lesser extent to phosphataemia. FGF23 is a phosphaturic hormone secreted by osteocytes and osteoblasts in response to an increase in phosphate uptake, to an increase in phosphataemia, or to an increase in serum calcitriol. Similar to PTH, FGF23 increases renal phosphate excretion by inducing the endocytosis and the degradation of sodium/phosphate co-transporters in the proximal tubule. FGF23 binds to FGF receptors whose specificity for FGF23 depends on the presence of the transmembrane protein αKlotho (here named Klotho). The Klotho protein is mainly expressed in the distal tubule, and to some extent in the proximal tubule [25]. Klotho, in addition to being a co-receptor for FGF23 activity, also has direct phosphaturic properties related to the activity of its cleaved form [26]. Interestingly, the absence of Klotho in mice recapitulates many of the extra-renal phenotype of CKD, and this has been related directly to phosphate retention [27, 28].

In response to phosphate retention due to decreased GFR, the FGF23 circulating level increases from the early stages of the disease, which leads to increased tubular phosphate excretion [29]. FGF23 also decreases 1,25-hydroxylation of vitamin D while increasing calcitriol catabolism, resulting in a decrease in circulating calcitriol levels [30]. PTH increases later than FGF23 during the course of CKD, in response to low calcitriol levels and hyperphosphataemia/hypocalcaemia. These compensatory mechanisms tend to maintain normal phosphataemia by increasing phosphate tubular excretion and decreasing intestinal absorption until the late stages of the disease. However, with advancing kidney disease, phosphate accumulation still occurs. Phosphate accumulation promotes cardiovascular disease by direct toxic effect on endothelial cells [31]. In addition, FGF23 elevation, although needed to control phosphataemia, is also known to directly induce cardiac hypertrophy [32, 33]. Finally, renal Klotho expression decreases early during CKD, leading to a resistance to FGF23, which participates in decreased phosphate excretion. In addition to phosphate retention, the decrease in the soluble Klotho, which possesses antiproliferative properties, may also promote cardiac hypertrophy and CKD progression [25, 34, 35].

Altogether, adaptations observed in CKD aim mainly to maintain normal phosphataemia. However, these mechanisms are not sufficient in advanced CKD, where phosphate accumulation occurs and participates in cardiovascular disease. Compensatory mechanisms, such as FGF23 and PTH elevation, although crucial to avoiding phosphate accumulation in early CKD stages, also promote heart hypertrophy and bone disease in CKD patients.

Modulating mineral metabolism and its effect on cardiac and renal disease

The majority of CKD patients will experience cardiovascular events, and eventually die before reaching end-stage renal disease. This observation leads us to consider cardiovascular disease as a high-priority problem in CKD. Although a large part of cardiovascular prevention will always rely on the control of traditional risk factors, such as diabetes and hypertension, some new avenues may open.

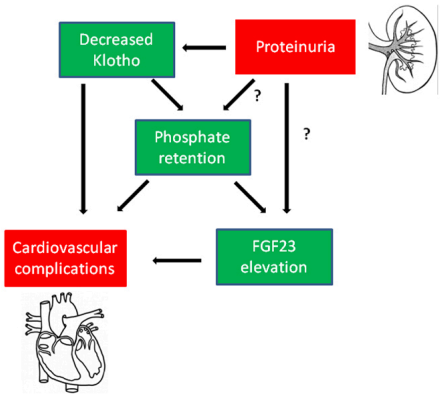
Maintaining normal phosphataemia is one of the current targets of nephrological care and relies mainly on the use of phosphate binders. However, this intervention is rather late and its efficiency still debated, whereas compliance is low due to the high number of pills needed. New leads include the decrease of gut phosphate absorption by transporter inhibitors such as tenapanor, which may increase patients' compliance by reducing the burden of pills needed. Experimentally, it has been demonstrated that neutralising anti FGF23-Ab in CKD rats increases mortality and aortic calcifications, potentially due to a dose-dependent increase in serum phosphate [36]. Targeting FGF23 to decrease heart hypertrophy may thus require more specific targets [33]. Therefore, another promising target in CKD seems to be the restoration of Klotho levels. Restoring Klotho levels, either by over-expressing the protein or administrating a soluble form of the protein, appears sufficient in experiments to slow renal disease progression, improve heart hypertrophy and calcifications, and decrease phosphataemia [37–39]. This may therefore become a novel therapeutic avenue in CKD patients. From this point of view, understanding Klotho regulation during early and late CKD is of crucial importance.

A potential link between albuminuria and phosphate retention

Given the important role of phosphate homeostasis during CKD, and the predictive role of albuminuria for cardiovascular events, we hypothesised in our work which received the Pfizer price and was published in the Journal of the American Society of Nephrology, that deficient phosphate excretion and albuminuria could be related [40].

We thus based our study on the hypothesis that albuminuria could be directly related to cardiovascular disease by promoting renal phosphate retention (fig. 1). This was motivated by recent observations showing that FGF23 levels are higher in children with glomerular (hence albuminuric) versus tubular disease [41]. In IgA nephropathy, FGF23 was also demonstrated to be higher in albuminuric patients

Figure 1: Pathway by which proteinuria increases cardiovascular mortality.



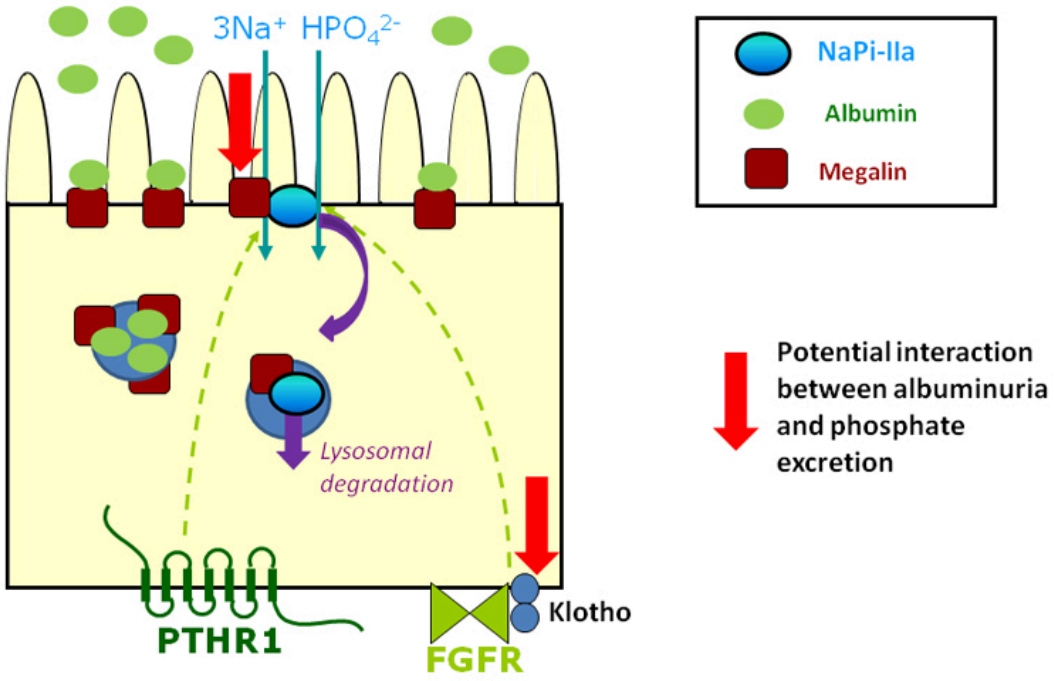
[42]. Quite interestingly, the predictive value of albumin and decreased eGFR for cardiovascular disease was no longer significant when correcting for elevation of FGF23 in the CRIC study of CKD patients, suggesting a link between albuminuria and FGF23 elevation [43]. At the molecular level, NPT2a endocytosis and subsequent lysosomal degradation requires a functional megalin/cubulin system, which is also used by albumin for endocytosis in the proximal tubule [44]. Finally Klotho down-regulation is described as an early event in CKD, long before renal function declines [45].

We therefore hypothesised that albuminuria could promote tubular phosphate retention independently of GFR decline, either by a competitive mechanism in the proximal tubule, or via Klotho regulation and a resistance to FGF23 action (fig. 2). To verify this hypothesis, we used different experimental approaches.

We first studied phosphate handling in children presenting acute nephrotic syndrome both in the nephrotic phase and in remission of the disease. We observed that, although eGFR was not altered in this setting, phosphate levels and FGF23 levels were higher during the nephrotic (proteinuric) phase compared to remission. We then studied the association between phosphataemia and albuminuria levels in a large cohort of CKD patients, the Nephrotest cohort in Paris. In 1,738 patients suffering from CKD at different stages, we did observe that the presence of albuminuria was associated with higher phosphate levels independently of other determinants for phosphataemia, including measured GFR. Circulating FGF23 concentrations, which were assessed in a subset of 397 patients, were also higher in the presence of albuminuria, independently of GFR. This implies that, despite higher FGF23, the presence of albuminuria is sufficient to impair renal phosphate excretion, leading to increased serum phosphate, and independently of renal function.

We further studied the molecular mechanisms of these observations *in vivo*, in two models of proteinuria in the rat and the mouse, as well as *in vitro* in cultured proximal tubule derived cells. Briefly, we demonstrated that proteinuric animals displayed higher levels of the sodium phosphate transporter NPT2a, despite higher FGF23 and PTH levels. The FGF23 downstream pathway was also down-regulated in the two models of proteinuric animals, suggesting a resistance to FGF23. This was seen together with a decrease in renal Klotho protein expression in both animal models. Finally, in cultured cells, albumin had no ap-

Figure 2: Molecular pathway linking albuminuria to phosphate retention. NaPi-II: sodium phosphate co-transporter type IIa, PTHR1: PTH receptor 1; FGFR: FGF receptor.



parent direct effect on phosphate transport. These experimental data confirmed a tubular impairment in phosphate excretion in the presence of albuminuria. The absence of albumin effect on cultured cells suggests that this defect does not rely on a direct competitive effect of albumin on phosphate absorption in the proximal tubule. Rather, albuminuria impaired FGF23 downstream pathway, probably due to a decrease in the Klotho protein, a major FGF23 co-factor and a regulator of phosphate excretion.

Altogether, these data confirm that modifications of albuminuria directly alter tubular phosphate handling by the kidney, leading to phosphate retention despite FGF23 elevation. Since both phosphataemia and FGF23 levels are associated with cardiovascular mortality, these observations substantiate a direct pathophysiological link between the presence of albumin in the urine, and the excessive cardiovascular mortality.

Perspectives

Our work has several strengths. We were able to confirm our hypothesis in the clinical setting, both in adults and children. We then used animals and cell models to study the pathways involved. These approaches confirmed the relevance of our observations.

Several questions remain unanswered and would deserve further study: Is the regulation of FGF23, Klotho and phosphate sufficient to explain the high cardiovascular mortality associated directly with albuminuria? This appears to be the case in the large CRIC study, but other cohorts would be necessary to confirm this data [43]. In children, several studies confirm our observation that nephrotic proteinuria is associated with higher phosphate levels [46]. Some studies further demonstrate that vascular rigidity is increased during childhood nephrotic syndrome [47]. The impact of such observations on later cardiovascular risk is however unknown. At the molecular level, the pathway by which albumin regulates Klotho protein expression also deserves further study. Indeed, targeting Klotho in albuminuric disease may be of great therapeutic interest.

Altogether, our published work links albuminuria to phosphate retention and FGF23 elevation [48] for the first time. It confirms that some of so called “biomarkers” of disease often play a crucial pathophysiological role, which explains their predictive value. Although more clinical and fundamental observations are needed to deepen these observations, we believe they may point to new therapeutic avenues in CKD.

Disclosure statement

No financial support and no other potential conflict of interest relevant to this article was reported.

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