In addition, sustained culture of renal-derived endothelial cells is typically difficult to attain. Compared with other endothelial cells cell types, kidney endothelial cells from rat grow slowly, have low colony-forming potential, and fail to form stable branched structures *in vitro*.[16] In our hands, both rat kidney endothelial cells[16] and mouse kidney endothelial cells (unpublished data) fail to proliferate in response to VEGF stimulation, despite a high level of VEGF-receptor expression.

In the current study by Kida *et al.*, although the proliferative and angiogenic response were reduced in the early phase of the UUO in ephrinB2 ΔV mice, the number of proliferating endothelial cells present even in wild type animals was modest. In this context, whether further reduction in the already nominal proliferation of kidney endothelial cells observed in ephrinB2 ΔV mice actually contributes to a greater degree of rarefaction is difficult to determine considering the multiple cell types being influenced by the mutations used in this study.

In recent years, several strategies have been proposed to overcome the vascular defects that develop after acute injury or during progression of CKD. These include the administration of exogenous vascular growth factors[15] or the use of hematopoietic derived proangiogenic cells,[17] also called endothelial progenitor cells.[18] In the kidney, these treatments may prevent, but have not been shown to reverse, capillary loss. The report provided by Kida *et al.* may help investigators shape appropriate questions in this area. The study provides a greater understanding of the intrinsic capacity of endothelial cells to undergo repair and the capacity of pericytes to stabilize nascent vascular cells. It also highlights the fact that the molecular interactions between these two cells types is an important determinant of the final structure generated in response to injury. By highlighting these points, the study is a welcome advance to an underappreciated problem.

DISCLOSURES

None.

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See related article, "EphrinB2 Reverse Signaling Protects against Capillary Rarefaction and Fibrosis after Kidney Injury," on pages 559–572.

Fibroblast Growth Factor-23 and Outcomes: New Answers, New Questions

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J Am Soc Nephrol 24: 523–525, 2013.
doi: 10.1681/ASN.2013020169

Even after taking into account comorbidities, such as a diabetes and hypertension, that often accompany CKD, patients are

Published online ahead of print. Publication date available at www.jasn.org.

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plagued by a high risk of early mortality, particularly because of cardiovascular disease (CVD).[1] Focus on traditional risk factors from the general population, such as control of serum lipids, has largely proven unfruitful.[2] This result has prompted a search for additional potentially modifiable factors that may be affected by CKD and contribute to the excess burden of mortality in this population.

One of the latest suspects in the search for these factors has been fibroblast growth factor-23 (FGF23). Despite its name, the primary action of FGF23 is not on fibroblast growth but phosphate homeostasis. This protein is expressed in bone and binds to an FGF/klotho receptor complex primarily in the kidney, where it acts to reduce both sodium-dependent phosphate reabsorption and the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D.[3] Although serum phosphate would seem to be a logical stimulus for FGF23 production, the hormone's regulation does not seem to be so straightforward, with possible contributions from 1,25-dihydroxyvitamin D, phosphate intake, bone mineralization, parathyroid hormone, and other factors.[4]

A steady drumbeat of studies has implicated FGF23 as a nefarious character in CKD. In a case control study of dialysis patients, members of our institution showed a stepwise increase in mortality risk with rising FGF23 levels, even among individuals with serum phosphate levels well within the normal range.[5] Indeed, the FGF23 effect was independent of a host of demographic and clinical factors, including serum phosphate. This finding has been now replicated by other groups,[6] and data from both early stage CKD and AKI have further linked FGF23 to both mortality and progression to dialysis.[7,8] Indeed, the association of FGF23 with mortality extends to patients with CVD and only mild reductions in GFR.[9]

FGF23 is a hormone: a substance produced by one organ (in this case, bone) that acts in a regulatory capacity at another site (*e.g.*, the kidney). With many hormones, absolute levels may not reflect their true activity. End organ resistance to the actions of a hormone may lead to a situation in which levels are normal or high, but activity is low. In this issue of *JASN*, Dominguez *et al.*[10] add a new wrinkle to the previous associations between FGF23 and outcomes by attempting to assess end organ activity.[10] Using their previously studied Heart and Soul cohort of 872 outpatients with stable CVD, Dominguez *et al.*[10] examine whether the effect of FGF23 on both overall mortality and CVD events (myocardial infarction, stroke, or CVD death) depends on the fractional excretion of phosphate. Given that a major known function of FGF23 is in its role as a phosphatonin (promoting the urinary wasting of phosphorus), fractional excretion of phosphorus (FePi) might serve as a measure of hormone action.

In straightforward multivariable analyses incorporating demographic factors and FePi, FGF23 was, as previously seen, associated with both mortality and CVD events. Interestingly, despite its potential use as a measure of FGF23 action, FePi had no such association. After finding statistical evidence for effect modification, however, Dominguez *et al.*[10] then put forth an easy to understand analysis. Using sample medians for both FGF23 and FePi, the population is divided into four groups: low FGF23 with low FePi, low FGF23 with high FePi, high FGF23 with low FePi, and high FGF23 with high FePi. Among subjects with low FGF23, the low and high FePi groups behaved identically, with no difference in either overall mortality or CVD events. Among patients with high FGF23, the effect was different. Subjects with high FGF23 but low FePi fared the worst, with the highest risk of poor outcomes. Those patients with high FGF23 but high FePi experienced intermediate outcomes: better than the remainder of subjects with high FGF23 but worse than patients with low FGF23. When examined by quartile of FGF23, the results are similar: a strong association between FGF23 and outcomes is noted among individuals with low FePi.

The study has many strengths, including a cohort large enough to tease out these complex interrelationships and the robustness of the findings. Neither phosphorus excretion nor FGF23 level is an independent factor, and indeed, both correlate with patient characteristics and renal function. To their credit, Dominguez *et al.*[10] provide multiple models that account for potential confounding factors, including demographic factors, such as age, sex, and race, as well as cardiovascular risk factors, such as diabetes, BP, cholesterol, and smoking. Although the precise effects of FePi varied depending on the multivariable model and many of the confidence intervals for these groups overlapped, the high FGF23–low FePi combination consistently displayed the poorest outcomes.

Although intriguing, there are, nonetheless, certain limitations of this study. The population was high risk: all patients had established CVD, and over one third of the patients died during the 7.5-year follow-up period. Despite these high-risk characteristics, most subjects had relatively preserved renal function at baseline, with a mean estimated GFR of 71 ml/min per 1.73 m^2. Although Dominguez *et al.*[10] provide evidence that this FePi remains an important effect modifier in moderate (stage 3) CKD, it is not clear how these results will apply, if at all, to patients with more advanced disease. Measures of renal function, FGF23, and phosphate excretion were all conducted at study onset. Change in these values over time and in particular, how measures of phosphorus excretion and FGF23 might predict subsequent decline in renal function remain issues for future studies.

Perhaps the most important role of this study is to begin to shed some light on potential underlying mechanisms that might be at play linking FGF23 to clinical effects. However, many more questions are raised that this study is unable to answer, particularly given the lack of clarity surrounding the regulation of FGF23 and even its potential mechanisms of toxicity. A natural question is what FePi represents independently from FGF23, which is considered to be the major hormonal regulator of such excretion, and CKD itself, which might limit the ability of FGF23 to act. An individual with high FGF23 but low FePi could be considered to have FGF23 resistance. Dominguez *et al.*[10] postulate that it may reflect decreased renal expression of klotho, which is necessary for the ability of FGF23 to promote phosphaturia. Although biopsy studies with detailed molecular analyses would be necessary to establish this definitively, it would remain uncertain how such a relationship would explain the mortality

findings in this study. If the effect of FGF23 resistance on mortality was simply mediated by FGF23 levels, then elevated FGF23 should have a consistent effect on mortality independent of FePi, but this result is not the case. Is phosphate the culprit? Dominguez *et al.*[10] did not specifically examine the relationship of phosphate with outcomes or control for phosphate levels in multivariable models. Prior studies, however, show the associations of FGF23 with mortality to be independent of phosphate. Although phosphate levels were highest in the group with high FGF23 and low FePi, mean levels were within the normal range in all groups. It remains possible, however, that the combination of FePi and FGF23 reflects a chronic phosphate burden not captured by isolated plasma measurements.

FGF23 may itself be toxic at high levels, which was suggested by a recent study showing a direct klotho-independent effect of FGF23 on cardiac myocytes.[11] One explanation to tie these results together is that FGF23 resistance takes time to develop and that the combination of high FGF23 and FePi reflects sustained exposure to high FGF23 levels. Alternatively, it may be that estimated GFR is an incomplete measure of kidney disease and that low FePi identifies subjects at risk for future progression. It is possible that FePi does, indeed, reflect klotho expression and that decreased availability of klotho sites allows for increased action of FGF23 on klotho-independent pathways, such as those pathways described in cardiac myocytes. Lastly, it remains possible that FGF23 is not a culprit itself but rather, a marker of underlying disease. Indeed, a recent study found that administration of FGF23 neutralizing antibodies in an animal model of CKD led to increased rather than decreased mortality.[12] Although this work by Dominguez *et al.*[10] cannot provide answers to sort through these possibilities, it does, as all important studies, prompt new questions to guide future studies into FGF23 biology.

DISCLOSURES

None.

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See related article, "Fractional Excretion of Phosphorus Modifies the Association between Fibroblast Growth Factor-23 and Outcomes," on pages 647–654.

Genetic Variants in Membranous Nephropathy: Perhaps a Perfect Storm Rather than a Straightforward Conformeropathy?

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J Am Soc Nephrol 24: 525–528, 2013.
doi: 10.1681/ASN.2013020166

Published online ahead of print. Publication date available at www.jasn.org.

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