Key role of the kidney in the regulation of fibroblast growth factor 23

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High circulating levels of fibroblast growth factor 23 (FGF23) have been demonstrated in kidney failure, but mechanisms of this are not well understood. Here we examined the impact of the kidney on the early regulation of intact FGF23 in acute uremia as induced by bilateral or unilateral nephrectomy (BNX and UNX, respectively) in the rat. BNX induced a significant increase in plasma intact FGF23 levels from 112 to 267 pg/ml within 15 min, which remained stable thereafter. UNX generated intact FGF23 levels between that seen in BNX and sham-operated rats. The intact to C-terminal FGF23 ratio was significantly increased in BNX rats. The rapid rise in FGF23 after BNX was independent of parathyroid hormone or FGF receptor signaling. No evidence of early stimulation of FGF23 gene expression in the bone was found. Furthermore, acute severe hyperphosphatemia or hypercalcemia had no impact on intact FGF23 levels in normal and BNX rats. The half-life of exogenous recombinant human FGF23 was significantly prolonged from 4.4 to 11.8 min in BNX rats. Measurements of plasma FGF23 in the renal artery and renal vein demonstrated a significant renal extraction. Thus the kidney is important in FGF23 homeostasis by regulation of its plasma level and metabolism.

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Uremia is characterized by high circulating levels of FGF23, an important risk factor associated with mortality, cardiovascular events, and progression to end-stage renal disease.[1–5] However, the mechanisms activating and regulating this large increase are not well understood.[6,7] FGF23 rises early in chronic kidney disease (CKD) in advance of plasma (p) phosphate (P), parathyroid hormone (PTH), and 1,25 dihydroxyvitamin D (1,25vit.D) derangements.[8–10] Recent studies have shown that FGF23 also increases in acute kidney injury (AKI) in both humans and experimental animal models.[10–13]

FGF23 is a 32-kDa glycoprotein secreted by osteocytes, which circulates as an active full-length protein and shorter, inactive fragments.[14–18] Only intact protein activates FGF receptors 1 (FGFR1), 3, and 4 in the presence of the required co-receptor klotho.[7] It decreases the type II sodium-dependent P co-transporters (NPT2a and NPT2c) in the proximal tubule of the kidney and thereby inhibits P reabsorption [19–21]. Furthermore, FGF23 reduces 1,25vit.D synthesis by inhibition of 25-hydroxyvitamin D 1-α-hydroxylase (Cyp27b1) and stimulation of 1,25vit.D 24-hydroxylase (Cyp24a1),[22,23] and hence acts as a counter-regulatory hormone to 1,25vit.D. FGF23 is thereby part of the complex endocrine network maintaining Ca^{2+} and P homeostasis, and classical negative feedback loops have been demonstrated between FGF23, 1,25vit.D and PTH.[7] In the bone, FGF23 is regulated by local bone factors and signaling through FGFR1.[24–28] 1,25vit.D has proved to be an important regulator of FGF23.[29–36] Ablation of FGF23 results in high 1,25vit.D levels, vascular calcifications, hypercalcemia, and hyperphosphatemia along with abnormalities in bone development and mineralization.[23,25]

FGF23 is an important regulator of Ca balance not only by regulation of 1,25vitD but also by stimulating Ca reabsorption in the distal renal tubule through TRPV5.[37–39] FGF23 has a principal role in P homeostasis, but it is unclear how a P load is sensed by the organism, and the question on how extracellular P regulates FGF23 expression is controversial.[38]

FGF receptors and klotho are expressed in the parathyroids with negative feedback loops created between FGF23 and PTH,[40,41] although the exact role of FGF23 in PTH regulation is not completely understood and vice versa.[42–49] An increase in PTH has been associated with a rise in FGF23 levels,[50,51]

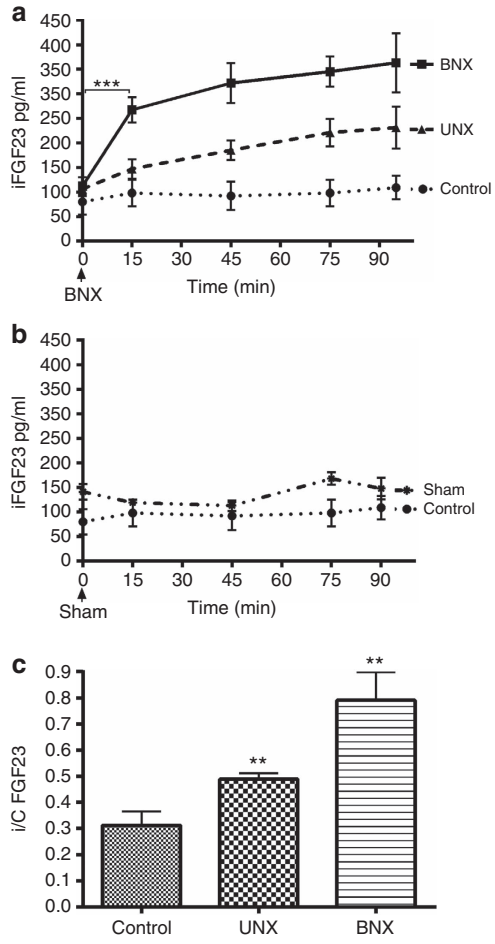

Figure 1 | Impact of acute bilateral (BNX) and unilateral (UNX) nephrectomy on plasma intact fibroblast growth factor 23 (iFGF23) and the ratio of intact-to-C-terminal FGF23. (**a**): Plasma levels of iFGF23 after acute BNX or UNX nephrectomy and in the control group. A rapid significant increase in p-iFGF23 took place within 15 min after BNX (***$P<0.01$). The following FGF23 levels remained stable. UNX generated a p-iFGF23 rise reaching a level in between the BNX and control group. BNX ($n=11$), UNX ($n=9$), and Control ($n=6$). (**b**) To examine whether the short-lasting acute kidney injury during the BNX procedure was responsible for the stimulated FGF23, a comparison between sham (see text) and control group was performed; however, similar p-iFGF23 levels were found ($n=6$). (**c**) The ratio of intact-to-C-terminal FGF23 was measured in the plasma of control, UNX, and BNX rats at 55 min after nephrectomy. A significant increase took place in UNX and further in BNX rats (**$P<0.05$) ($n=6$).

and a direct stimulatory effect of PTH on activation of protein kinase A (PKA) and wnt signaling has been demonstrated in osteoblast-like cells.[40,51–55]

The purpose of the present *in vivo* study in the rat was to examine the influence of the presence of kidneys on the very early regulation of FGF23 in a model of acute uremia induced by bilateral or unilateral nephrectomy (BNX and UNX, respectively) and to examine the impact of acute hyperphosphatemia, acute hypercalcemia, PTH, and the FGFR signaling pathways on the metabolic response of FGF23 to acute uremia.

RESULTS

Effect of acute BNX on plasma FGF23 levels

Acute BNX resulted in an immediate significant increase in p-FGF23 from 112 ± 18 to 267 ± 26 pg/ml ($P<0.001$), taking place within 15 min after BNX (Figure 1a). The following high FGF23 levels remained stable. UNX also generated a rapid rise of p-FGF23 to 147 ± 20 pg/ml reaching p-FGF23 levels between BNX and control (Figure 1a). To examine whether the short-lasting kidney injury during the BNX procedure stimulated FGF23, a comparison between sham-operated and control rats was performed, which demonstrated similar levels (Figure 1b). Although p-P levels were unchanged after acute BNX (2.32 ± 0.12 vs. 2.20 ± 0.12 mmol/l, not significant), a significant drop in p-Ca^{2+} was measured from 1.41 ± 0.01 to 1.36 ± 0.01 mmol/l ($P<0.01$). Both p-Ca^{2+} and p-P were stable in UNX, sham, and control rats (data not shown). In order to examine whether acute uremia is associated with increased secretion of intact FGF23 (iFGF23), the intact-to-C-terminal FGF23 ratio was measured in control, UNX, and BNX rats in samples obtained 55 min after the nephrectomy. The intact/C-terminal FGF23 ratio was significantly increased in the UNX group ($P<0.05$) and increased further in the BNX group ($P<0.05$) (Figure 1c).

To determine whether PTH signaling was involved in the increase of p-FGF23 after BNX, total parathyroidectomy was performed prior to BNX (PTX-BNX). However, similar p-FGF23 levels were found in the PTX-BNX and BNX groups (Figure 2). A significant fall in p-Ca^{2+} was also demonstrated after BNX in the PTX-BNX group from 1.38 ± 0.01 to 1.30 ± 0.01 mmol/l ($P<0.05$), and p-P was stable until 45 min after BNX (Supplementary Figure S1 online). PTX alone had no effect on p-FGF23, and its p-FGF23 levels were similar to those of sham group (Figure 2).

The potential involvement of FGFR signaling in FGF23 regulation was studied using a pharmacological inhibitor of FGFR (PD173074) administered to normal and BNX rats prior to BNX. We found that the dose of 40 mg PD173074 would induce maximal suppression of FGF23 biosynthesis and secretion at 5 h (Figure 3). In addition to the suppressed p-FGF23, the bone's expression of FGF23 gene was reduced by one-half (Figure 3b). After prior FGFR inhibition, BNX resulted in an increase of p-FGF23 to 52 ± 9 pg/ml in comparison to vehicle group (182 ± 100 pg/ml) after BNX (Figure 4). The percentage of increases were, however, not significantly different and do not indicate an involvement of the FGFR signaling pathway.

As the large increase in p-FGF23 took place within only 15 min, it would unlikely be due to increased transcription of FGF23 in bone cells, yet FGF23 gene activity in bone was further studied but demonstrated similar FGF23 mRNA expression levels in the BNX versus control group (Figure 4b).

FGF23 metabolism

In order to evaluate the importance of kidneys on FGF23 metabolism, disappearance of recombinant human FGF23 (rhFGF23) was followed in normal and BNX rats.