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BIOLOGICAL ACTIVITY OF FGF-23 FRAGMENTS

Theresa J. Berndt[1,2], **Theodore A. Craig**[1], **Daniel J. McCormick**[3], **Beate Lanske**[4], **Despina Sitara**[4], **Mohammed S. Razzaque**[4], **Marlon Pragnell**[5], **Ann E. Bowe**[5], **Stephen P. O'Brien**[5], **Susan C. Schiavi**[5], and **Rajiv Kumar**[1,3]

[1]Department of Internal Medicine, Mayo Clinic College of Medicine, Mayo Clinic Rochester, 200 First Street SW, Rochester, MN 55905, USA

[2]Department of Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Mayo Clinic Rochester, 200 First Street SW, Rochester, MN 55905, USA

[3]Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Mayo Clinic Rochester, 200 First Street SW, Rochester, MN 55905, USA

[4]Department of Developmental Biology, Harvard School of Dental Medicine, 188 Longwood Ave., Boston, MA 02115

[5]Receptor Ligand Therapeutics, Endocrine and Renal Sciences, Genzyme Corp., 1 Mountain Rd, Framingham, Massachusetts 01701, USA

SUMMARY

The phosphaturic activity of intact, full-length, fibroblast growth factor-23 (FGF-23) is well documented. FGF-23 circulates as the intact protein, and as fragments generated as the result of proteolysis of the full-length protein. To assess whether short fragments of FGF-23 are phosphaturic, we compared the effect of acute, equimolar infusions of full-length FGF-23 and various FGF-23 fragments carboxyl-terminal to amino acid 176. In rats, intravenous infusions of full-length FGF-23, and FGF-23 176-251, significantly and equivalently increased fractional phosphate excretion (FE Pi)) from 14±3 to 32±5% and 15±2 to 33±2% ($p < 0.001$), respectively. Chronic administration of FGF-23 176-251 reduced serum Pi and serum concentrations of 1α,25-dihydroxyvitamin D. Additionally, FGF-23 176-251 reduced serum Pi when administered intraperitoneally to hyperphosphatemic *Fgf23*$^{-/-}$ mice. Shorter forms of FGF-23 (FGF-23 180-251 and FGF-23 184-251) retained phosphaturic activity. Further shortening of the FGF-23 carboxyl-terminal domain, however, abolished phosphaturic activity, as infusion of FGF-23 206-251 did not increase urinary phosphate excretion. Infusion of a short fragment of the FGF-23 molecule, FGF-23 180-205, significantly increased FE Pi in rats and reduced serum Pi in hyperphosphatemic *Fgf23*$^{-/-}$ mice. The activity of FGF-23 180-251 was confirmed in OK cells in which the peptide reduced Na^+-dependent Pi uptake and enhanced internalization of the Na^+-Pi IIa co-transporter. We conclude that carboxyl terminal fragments of FGF-23 are phosphaturic, and that a short, 26 amino acid fragment of FGF-23 retains significant phosphaturic activity.

Keywords

FGF-23; rat; phosphate; kidney; 1α,25$(OH)_2$D

Address correspondence to: Rajiv Kumar, MS 1-120, Mayo Clinic Rochester, Mayo College of Medicine, 200 First Street SW, Rochester, MN 55905, USA, Phone 507-284-0020, Fax 507-538-3954, rkumar@mayo.edu.

INTRODUCTION

Fibroblast growth factors (FGF) are involved in many biological processes including embryonic development and cellular homeostasis in the adult [1, 2]. The twenty three FGFs and the four known plasma membrane associated receptors, the fibroblast growth receptors (FGFRs) transduce a variety of biochemical changes in developing and adult tissues [3-5]. The recently identified fibroblast growth factor, FGF-23, inhibits renal phosphate reabsorption and 1α,25-dihydroxyvitamin D (1α,25$(OH)_2$D) synthesis [6-13]. FGF-23 plays a role in the pathogenesis of a variety of hypophosphatemic disorders including autosomal dominant hypophosphatameic rickets, tumor-induced osteomalacia and X-linked hypophosphatemic rickets [14-16]. Recently, the autosomal recessive disorder, familial tumoral calcinosis (FTC), characterized by ectopic calcifications and elevated serum phosphate levels, has been associated with a homozygous missense mutation in the FGF23 gene [17]. FGF-23 is expressed at high levels in the brain and bone [18, 19], and in low levels in a variety of tissues [6, 20]. The protein is synthesized as a 251-amino acid precursor protein with a 24 residue signal peptide. The role of the protein in phosphate homeostasis has been explored following the administration of the peptide to transporting epithelial cells [8] and mice [15, 21, 22], by the transgenic overexpression of the protein [23-25] and gene ablation experiments [18, 26, 27]. These experiments and several other recent *in vivo* studies demonstrate that FGF-23 is a phosphaturic factor and counter-regulates 25-hydroxyvitamin D 1α-hydroxylase expression.

In autosomal dominant hypophosphatemic rickets (ADHR), a disease associated with low serum phosphorus and 1α,25$(OH)_2$D concentrations and rickets, mutations in *FGF-23* gene result in the expression of a protein that is resistant to proteolysis and with an increased half life [7, 9, 11, 14, 28]. Mutations in the gene alter the furin proconvertase recognition site (176RHTR179) in the protein such that the arginine residue at 176 is replaced by glutamine (R176Q) or the arginine residue at amino acid 179 is replaced by a glutamine (R179Q) or tryptophan residue (R179W). In contrast, individuals with tumoral calcinosis have elevated serum phosphate levels and elevated serum levels of the FGF-23 carboxyl terminal fragments [17, 29]. Shimada *et al* have tested the bioactivity of a biosynthetic carboxyl terminal fragment of FGF-23 (aa 180-251) and an N-terminal fragment of FGF-23 (25-179) and have shown that in contrast to the phosphaturic action of the full-length protein, these peptides fragments are biologically inactive 24 hours after their administration intraperitoneally to mice [11] at a dose of 5 γg per mouse every 12 hours. These results suggest that the processing of FGF-23 R179 and S180 may abolish the phosphaturic activity of FGF-23. Alternatively, differential processing of the fragments and/or changes in serum half-life could obscure potential biological actions.

To more completely assess the biological activity of carboxyl terminal FGF-23 peptides, we performed solid phase peptide synthesis of FGF-23 carboxyl terminal fragments and compared their acute phosphaturic activity to that of full-length FGF-23. We now demonstrate that FGF-23 176-251, 180-251, 184-251 and 180-205 retain significant phosphaturic activity in rats and mice. The shortest fragment of FGF-23, FGF-23 180-205 can effectively lower serum Pi concentrations in *Fgf-23*$^{-/-}$ mice. These results suggest that residues 176 to residue 206 are important for transducing the phosphaturic activity. Since these peptides are easily synthesized they can be used to assess FGF-23 function instead of the native full-length protein that is difficult to synthesize in large amounts and is readily degraded. Furthermore, they can potentially be used for the treatment of disorders associated with hyperphosphatemia.

RESULTS

All proteins and peptides were of the appropriate molecular mass (see Figure 1). Data for groups 1-7, performed to determine the effect of the acute intravenous infusion of equimolar amounts of FGF-23 fragments in normal rats are summarized in Table 1. The glomerular filtration rate (GFR) was stable throughout the experiment in all groups studied. In the vehicle-infused group, the fractional excretion of sodium (FE Na) and fractional excretion of phosphate (FE Pi) were stable throughout the experiment (Figure 2). The acute intravenous infusion of full length recombinant FGF-23 significantly increased the FE Pi from 14±3 to 32±5% ($p<0.001$) and FE Na from 0.3±0.1 to 0.7±0.2% ($p = 0.01$). Infusion of FGF-23 176-251 significantly increased the FE Pi from 15±2 to 33±2% ($p<0.0001$) and the FE Na from 0.90±0.15 to 1.21±0.17 ($p = 0.020$. Infusion of FGF-23 180-251 significantly increased the FE Pi from 14±3 to 26±3% ($p = 0.0004$) and FE Na from 0.20±0.07 to 0.80±0.17% ($p = 0.014$). Infusion of FGF-23, 184-251 significantly increased FE Pi from 16±3 to 28±3% ($p = 0.0006$) and FE Na from 0.3±0.1 to 0.6±0.16%, $p<0.05$ ($p = 0.006$). Likewise, infusion of FGF-23 180-205 significantly increased FE Pi from 10±2 to 20±2% ($p = 0.004$) and FE Na from 0.4±0.1 to 1.0±0.2%, ($p = 0.03$). In contrast, infusion of FGF-23, 206-251 did not increase either phosphate or sodium excretion.

The magnitude of the increase in phosphate excretion was greatest in the full length FGF-23 and FGF-23 176-251. However, FGF-23 fragments 180-251, 180-205 and 184-251 also increased phosphate, as well as sodium excretion.

Chronic (72 Hr) administration of FGF-23 176-251 or vehicle resulted in serum phosphate concentrations of 1.85±0.12 mM and 2.19±0.11 mM ($p=0.062$). FGF-23 176-251 treatment significantly decreased serum $1\alpha,25(OH)_2D$ concentrations (76±11pg/mL) compared to the vehicle treated rats (114±7 pg/mL, $p < 0.05$). FGF-23 176-251 given intraperitoneally also reduced serum Pi concentrations in *Fgf-23*$^{-/-}$ mice (data not shown).

To further examine the *in vivo* phosphaturic activity of the smallest FGF-23 fragment, we injected 0.2 nmoles of FGF23 180-205, twice intraperitoneally into *Fgf-23*$^{-/-}$ mice; FGF-23 R176Q protein was similarly injected to the mutant mice as positive control, and saline as vehicle control. The results show that the C-terminal FGF-23 fragment of 180-205 was bioactive, as reflected by a significant decrease in serum phosphate levels in *Fgf-23*$^{-/-}$ mice (14.64 ± 0.51 pre-injected vs. 10.95 ± 0.62 post-injected; $p< 0.01$) (Figure 3). These data suggest that the C-terminal fragment of the FGF-23, containing only amino acids 180-205, could regulate phosphate homeostasis.

FGF23 or FGF23 180-251 directly inhibited radiolabeled phosphorus uptake in the standard *in vitro* assay using opossum proximal tubular epithelial cells (OK) (Figure 4). To assess the mechanism by which these agents altered phosphate transport, we examined the surface expression of an epitope tagged sodium phosphate transporter, NaPi IIa-V5. Immunofluorescent data confirmed that the anti-V5 antibody readily detected plasma membrane localized NaPi IIaV5 whereas there was no significant staining in the absence of the primary antibody (Figure 5a and 4d). Three hour treatment of FGF23 or FGF23 180-251 resulted in significant decreases in the amount of NaPiIIa-V5 detected (Figure 5b-c). Similar decreases in immunodetection of NaPiIIa-V5 occurred after incubation with PTH 1-34 (Figure 5e) but not the inactive PTH 13-34 (Figure 5f).

DISCUSSION

The present studies demonstrate that acute intravenous infusions of equimolar doses of full length FGF-23 and FGF-23 176-251 result in similar increases in phosphate excretion. Infusion of FGF-23 fragments 180-251, 180-205 and 184-251 also significantly increase

phosphate excretion, although the magnitude of the increase in phosphate excretion is less than the phosphaturic response observed with infusion of full length FGF-23 or FGF-23 fragment 176-251. Of interest, these bioactive fragments also increase sodium excretion when administered over the short-term. This is very likely due to the movement of sodium ions along with phosphate in the proximal tubule. With long term-administration the sodium wasting is no longer seen, perhaps due to increased sodium reabsorption in the distal tubule.

The effects of the acute infusion of full length FGF-23 on phosphate excretion are consistent with the *in vivo* studies by Shimada *et al* and Schiavi *et al* in mice which demonstrate that administration of recombinant full length FGF-23 increases phosphate excretion [11, 15, 21, 22]. However, in contrast to subsequent studies by Shimada *et al* in which neither the N-terminal or C-terminal fragments of FGF-23 affected serum phosphate or phosphate excretion, in the present study, we show that the infusion of the carboxyl fragments of FGF-23 176-251, 180-205, 184-251, and 180-251 all significantly increase phosphate [11]. Of the peptides tested, only FGF-23 206-251 did not exhibit any biologic activity. The precise reason for the differences in bioactivity of the fragments tested by us compared to those tested by Shimada *et al* is not known [11].

To validate our *in vivo* observations that various C-terminal fragments are bioactive, we also injected the smallest C-terminal fragment (180-205) into *Fgf-23*$^{-/-}$ mice, and found a significant decrease in serum phosphate levels when compared to vehicle-injected *Fgf-23*$^{-/-}$ mice [18, 30]; these results suggest that C-terminal fragments could alter phosphate homeostasis in *Fgf-23*$^{-/-}$ mice, and that only a small part of the FGF23 protein has the potential to be used for treatment of diseases with hyperphosphatemia.

The *in vitro* studies demonstrate that similar to PTH, FGF23 and FGF23 180-251 inhibit sodium-phosphate transport by reducing the amount of plasma membrane localized NaPi IIa. FGF23 contains a consensus binding domain for FGFRs and several studies have suggested that FGF23 may bind and activate signaling through one or more FGFRs [31-33]. Recent *in vivo* genetic manipulation studies suggest that both FGF23 and KLOTHO act through a common signaling pathway [30] and that KLOTHO facilitates FGF23 binding to an FGFR [34]. Of relevance is the absence of the canonical FGFR binding domain on the bioactive FGF23 180-251 fragment. These results raise the possibility that FGF23 may bind to a novel receptor, in addition to a known FGFR.

The role of FGF-23 in the pathogenesis of ADHR has been shown to be due to mutations in the FGF-23 gene that result in the production of a mutant protein in which the amino acid sequence of a furin proconvertase cleavage site (176RHTR179) is altered (R176Q, R179Q, R179W), rendering the mutant FGF-23 resistant to proteolysis[6, 7, 9]. The mutant FGF-23 has a prolonged half-life [6, 7, 9, 11, 28]. Our studies suggest that although furin cleavage between residues 176 and 179 results in a reduction of bioactivity of FGF-23, significant bioactivity persists with fragments that extend from carboxyl terminal amino acid residue 180 up to residue 206. This, in turn, suggests that further cleavage of FGF-23 in the carboxyl terminal domain needs to occur in order render the molecule inactive. Indeed, Campos and others have demonstrated the presence of PHEX cleavage sites at amino acid residue 183 and 215 [35]. Potential PHEX cleavage sites also exist at residues 186, 188 and 208, all of which may play a role in altering the bioactivity of the FGF-23. Since the 180-205 fragment of FGF-23 is bioactive, it is likely that sites carboxyl-terminal of residue 205, are important in the proteolytic processing and bio-inactivation of FGF-23.

It is interesting to note that patients with tumoral calcinosis have elevated concentrations of FGF-23 measured by an assay that detects carboxyl terminal fragments of FGF-23 [17, 29]. In contrast, these patients have normal or low-normal concentrations of intact FGF-23. Our

data would suggest that the carboxyl terminal fragments circulating in patients with tumoral calcinosis are different than those tested by us. Alternatively, if FGF-23 fragments found in the circulation of patients with tumoral calcinosis are similar to those tested by us, they must be present in concentrations considerably lower than those achieved in our experiments.

In conclusion, we have identified the phosphaturic, bioactive domain of FGF-23 and show that it is present in the 176-205 region of the protein.

MATERIALS AND METHODS

Protein and Peptide Synthesis

Synthesis of full-length recombinant 25-251 human FGF-23 was carried out using bacterial protein expression methods. 25-251 human FGF-23 was expressed in pET28a(+), *E. coli* Rosetta 2(DE3) cells (Novagen/EMD, San Diego, CA), at 20 °C in the presence of the inducer, isopropyl β-D-thiogalactoside (IPTG) (0.1 mM). Cells were lysed at 4°C in lysis buffer (20 mM Na_2HPO4, 1.0 M NaCl, 10 mM beta-mercaptoethanol, pH 7.0) with 4 mM phenylmethanesulfonyl fluoride (PMSF) using an ice jacketed Bead Beater (Biospec Industries, Inc., Bartlesville, OK; 8 cycles of 20 sec on/ 2 min off). The cell lysate was centrifuged at 4 °C at 20,000 x g for 30 min. The supernatant was stirred at 4 °C with 15 ml nickel chelating sepharose (Amersham/GE Healthcare Bio-Sciences Corp., Piscataway, NJ) for 2 hr. The resin was pelleted by centrifugation, extensively washed, and then finally washed on a column with 1 liter lysis buffer at 4 °C over 12 hr. Protein was eluted with lysis buffer containing 1 M imidazole (80 ml) and dialyzed against lysis buffer before being loaded onto a 5 ml HisTrap column (Amersham/GE Healthcare). Protein was eluted by an imidazole gradient (0-1 M) in lysis buffer. Fractions were analyzed by SDS-PAGE and Coomassie blue/silver stain and by immunoblots (PhastTransfer) (Amersham/GE Healthcare) using an affinity purified polyclonal antibody against hFGF23, and goat anti-rabbit HRP secondary antibody (Dako, Carpinteria, CA), visualized by chemiluminescence (Roche, Indianapolis, IN). Fractions containing N-terminal 6x His 25-251 hFGF23 were combined and dialyzed against 20 mM Na_2HPO_4, 5 mM beta-mercaptoethanol, pH 7.0, and loaded onto an HR5/5 Mono S column (Amersham/GE Healthcare) and eluted with a NaCl gradient (0-1 M). Mono S fractions were analyzed by SDS-PAGE/immunobloting. Fractions containing highly purified 25-251 hFGF23 (~0.25 M NaCl) were pooled and further analyzed by N-terminal protein sequencing. 25-251 FGF23 was dialyzed against N_2-purged 0.9 % NaCl at 4 °C before animal infusions.

The synthesis of five FGF-23 peptides (*cf.* Figure 1) was performed in the Mayo Peptide Synthesis Facility by solid phase methods on an ABI 433A peptide synthesizer (Applied Biosystems Inc., Foster City, CA) using protocols previously described for the synthesis of human amyloid-β peptides [36]. Briefly, FGF-23 peptides (0.1 mmol scale) were synthesized on NovaSyn TGA resin (Calbiochem-Novabiochem, San Diego, CA) using HBTU activation of N^{α}-9-fluoroenylmethoxy-carbonyl (Fmoc) amino acid derivatives and synthesis protocols provided by the instrument's manufacturer. After completion of synthesis, all peptides were cleaved from the TGA resin support using 10 ml of a solution of 87.5% trifluoroacetic acid (TFA), 5% water, 5% phenol, and 2.5% triisopropylsilane (v/v/wt/v) for 2h at 22°C. The cleaved peptides were then precipitated in 40 ml cold tert-butylmethyl ether and purified by reverse phase HPLC on a C_{18} Jupiter column (250 mm × 21.2 mm, Phenomenex Corp.) using a binary gradient of 0.1% aqueous TFA containing 5% acetonitrile (buffer A) and 0.1% aqueous TFA containing 80% acetonitrile (buffer B). The calculated mass for each FGF-23 synthetic peptide was verified by electrospray ionization mass spectrometry using a MSQ single quadrupole mass analyzer (Thermo Electron Corp., San Jose, CA) using the following parameters: start *m/z* of 500, stop *m/z* of 2000, dwell time

of 0.1 msec at 40 scans/min. Each spectrum was deconvoluted into a single mass using Bioworks Browser provided by Thermo Electron.

Animals

All animal protocols were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Male Sprague Dawley rats weighing 300 g were purchased from Harlan Sprague Dawley Inc. (Madison WI, USA). They were fed a standard rodent diet containing 0.7% phosphate and 0.5% calcium. All animals were fasted overnight prior to the experiment. On the day of the experiment, rats were anesthetized with an intraperitoneal injection of 100-150 mg/kg body weight of 5-sec-butyl-ethyl-2-thiobarbituricacid (Inactin, Byk Gulden Konstanz, Hamburg Germany). The rats were placed on a heated table to maintain body temperature between 36-38 °C. After a tracheostomy, a PE 50 catheter was placed in the left carotid artery to monitor mean arterial blood pressure (MAP) and to collect blood samples. Catheters were also placed in the left and right jugular veins for infusions. 2% inulin in 0.9% NaCl plus 4.5% BSA were infused at a rate of 1.2 mL/Hr in one catheter and 0.9% NaCl was infused in the other catheter at a rate of 1.2 mL/Hr. A catheter (PE 90) was placed in the bladder for urine collection. Seven groups of animals were studied.

Acute Infusion Studies

Group 1—Vehicle Time control (n=8). After a ninety-minute recovery period, one thirty-minute urine sample was taken (C1) and a blood sample was taken. One hour later a second 60- minute clearance was taken (C2).

Group 2—Effect of acute infusion of full length FGF-23 (1.24 nmol/kg/Hr, n=8). This protocol is identical to group 1 except that after the control collections, full-length recombinant FGF-23 was added to the 0.9% saline infusion to deliver 1.24 nmol/kg/Hr of full length FGF-23. After one hour of infusion, a sixty- minute clearance was taken.

Group 3—Effect of acute infusion of FGF-23, 176-251 (1.24 nmol/kg/Hr, n=8). This protocol is identical to group 2 except for the sequence of the infused peptide.

Group 4—Effect of acute infusion of FGF-23, 180-251 (1.24 nmol/kg/Hr, n=8). This protocol is identical to group 2 except for the sequence of the infused peptide.

Group 5—Effect of infusion of FGF-23, 184-251 (1.24 nmol/kg/Hr, n= 8). This protocol is identical to group 2 except for the sequence of the infused peptide.

Group 6—Effect of infusion of FGF-23, 206-251 (1.24 nmol/kg/Hr, n=6). This protocol is identical to group 2 except for the sequence of the infused peptide.

Group 7—Effect of infusion of FGF-23, 180-205, 1.24nmol/kg/Hr, n=6). This protocol is identical to group 2 except for the sequence of the infused peptide.

Chronic Studies

These studies determined the effect of the chronic (72 Hr) administration of FGF-23 176-251 (1.24nmol/kg/hr, n=5) or vehicle (PBS +0.1% BSA, n=6) on serum phosphate and 1α, 25 $(OH)_2D$ concentrations. Rats received an intraperitoneal injection of FGF-23 176-251 in 0.5mL of normal saline every 12 hours for 3 days. At the end of three days, the animals were anesthetized and a terminal blood sample was taken.

Injection of FGF 23 R176Q and FGF23 180-205 into Fgf-23$^{-/-}$ mice

Fgf-23$^{-/-}$ animals were generated as reported earlier [18]. To determine the *in vivo* effects of the C-terminal fragment FGF23 180-205 in the absence of endogenous Fgf-23 activity, equimolar amounts (0.2nmoles) of FGF23 R176Q, FGF23 180-205, and vehicle (saline) were injected twice intraperitoneally into *Fgf-23*$^{-/-}$ mice at 0 and 8 hours. Blood was obtained by tail bleeding or cheek pouch puncture of 3- to 6-week-old *Fgf-23*$^{-/-}$ animals, 8 hours after the second injection (at 16 hours). Serum levels of phosphate were determined in pre-and post-injected mice using the Stanbio LiquiUV kit (Stanbio Laboratory, Boerne, TX),

OK Cell Pi Uptake

This was performed as described by Bowe *et al* [8].

Generation of Stable OK Cell Lines Expressing a V5-tagged Na$^+$-Pi IIa Transporter

The cell line was generated as described earlier [37].

Immunohistochemistry of OK- Na$^+$–P$_i$-IIa-V5 cells

The presence of the transporter on the cell surface was carried out using methods and reagents described earlier [37].

Analytical Methods

Plasma and urine phosphate concentrations were determined using the method of Chen [38]. Inulin concentrations in plasma and urine were determined using the anthrone method [39]. Sodium concentrations in urine were determined by ion selective electrode (EasyLyte Plus Analyzer, Medica Corp., Bedford, MA 01730). Serum 1α,25(OH)$_2$D concentrations were measured using an RIA kit (Diasorin, Stillwater, MN).

Statistical Analysis

Statistically significant differences between groups were evaluated by Student's t-test for comparison between two groups or by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. All values were expressed as mean ± SE. A p value less than 0.05 was considered to be statistically significant.

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Sequence and Molecular Weights of FGF-23 Peptides		
Name of Peptide	**Amino Acid Sequence**	**Molecular Weight**
Full-length FGF-23 25-251	25 ypnasp llgsswggli hlytatarns yhlqihkngh vdgaphqtiy salmirseda gfvvitgvms rrylcmdfrg nifgshyfdp encrfqhqtl engydvyhsp qyhflvslgr akraflpgmn pppysqflsr rneiplihfn tpiprrhtrs aeddserdpl nvlkprarmt papascsqel psaednspma sdplgvvrgg rvnthaggtg pegcrpfakf i 251	25331 With tag: gsshhhhhhs sglvprgshm asmtggqqmg rgs from construct 28744
FGF-23 176-251	176 rhtrs aeddserdpl nvlkprarmt papascsqel psaednspma sdplgvvrgg rvnthaggtg pegcrpfakf i 251	8070
FGF-23 180-251	180 aeddserdpl nvlkprarmt papascsqel psaednspma sdplgvvrgg rvnthaggtg pegcrpfakf i 251	7519
FGF-23 184-251	184 dserdpl nvlkprarmt papascsqel psaednspma sdplgvvrgg rvnthaggtg pegcrpfakf i 251	7117
FGF-23 206-251	206 csqel psaednspma sdplgvvrgg rvnthaggtg pegcrpfakf i 251	4714
FGF-23 180-205	180 aeddserdpl nvlkprarmt papas 205	2824

Figure 1.
chematic representation of the structure of full length FGF-23 and FGF-23 fragments.

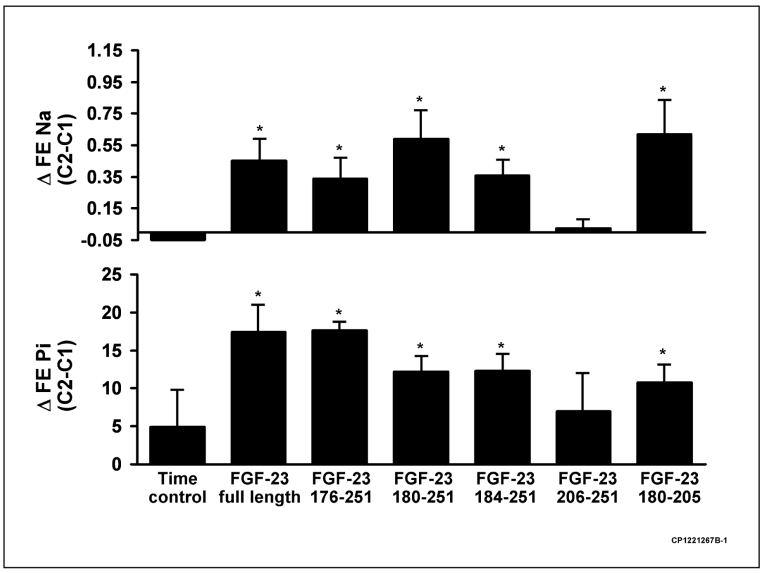

Figure 2.

Bioactivity of various FGF-23 peptides. Effect of full length recombinant FGF-23 and FGF-23 fragments 176-251, 180-251, 184-251, 206-251 and 180-205 on the excretions of phosphate (FE Pi) and sodium (FE Na). The solute excretion in period C1 (control) was subtracted form the value in the experimental period (C2). An * indicates a significant difference, C 1 compared to C 2, paired T test, $p<0.05$. Data are expressed as mean±SEM.

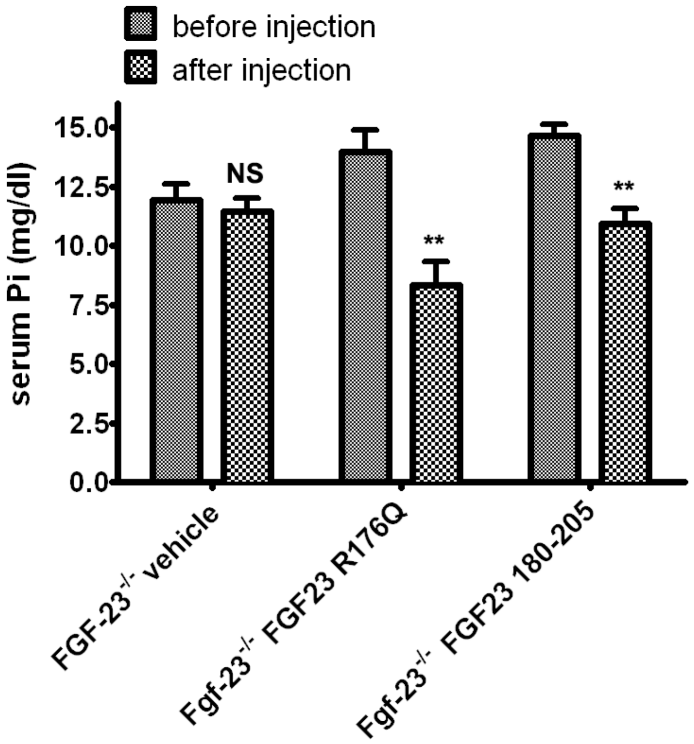

Figure 3.
Serum phosphate levels of *Fgf-23*$^{-/-}$ mice before and after ip injection of vehicle (saline), FGF23 R176Q, and FGF23 180-205. NS = not significant, ** = P<0.01

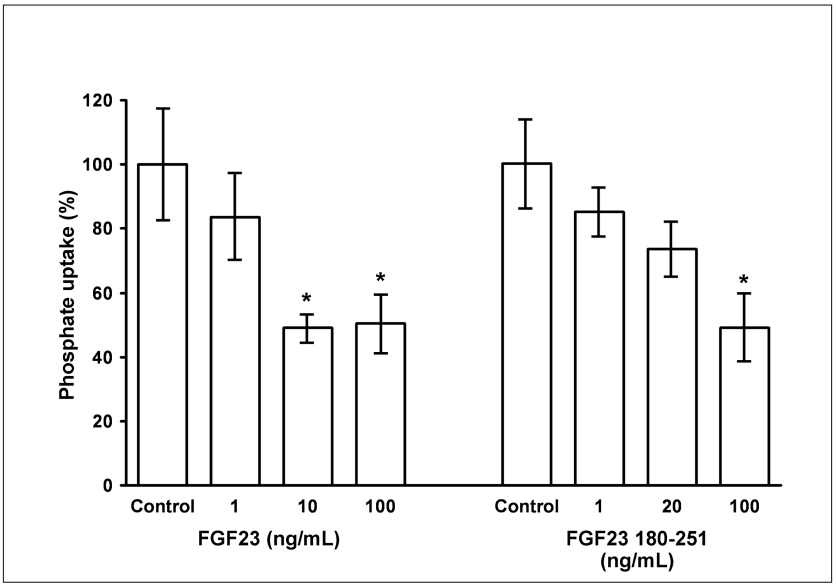

Figure 4. Inhibition of Sodium-dependent Phosphate Transport in OK Cells by FGF-23 and FGF-23 180-251

Effect of FGF-23 or FGF-23 180-251 on sodium-dependent phosphate transport in opossum kidney cells. An * indicates statistically significant differences, experimental vs. control, non-paired T test, $p<0.05$. Data are expressed as mean±SEM.

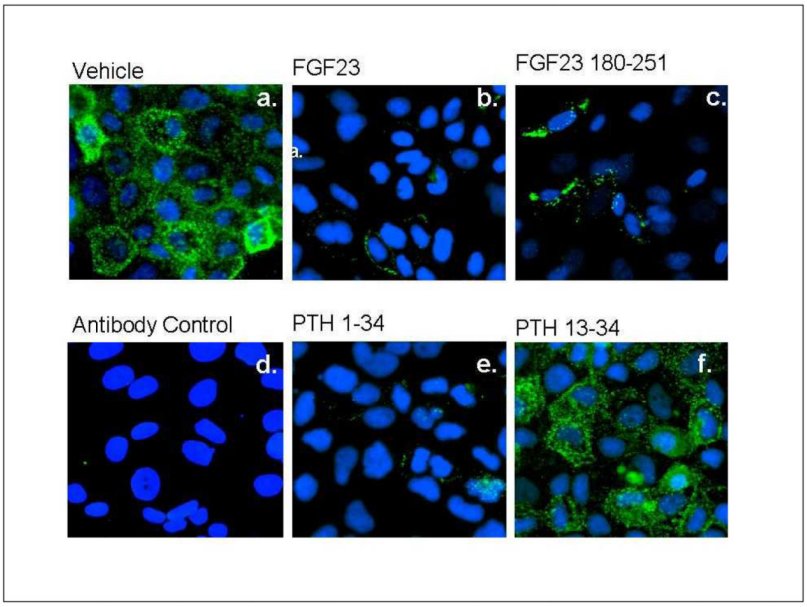

Figure 5.
Internalization by FGF-23 or FGF-23 180-251 of the sodium-dependent co-transporter IIa in OK cells.

Table 1

EFFECT OF INFUSION OF EQUIMOLAR AMOUNTS OF FGF-23 FRAGMENTS ON RENAL FUNCTION

Effect of full length recombinant FGF-23 and FGF-23 fragments 176-251, 180-251, 184-251, 206-251 and 180-205 on the fractional increases in the excretions of phosphate (FE Pi) and sodium (FE Na).

	GFR (mL/min)		FE_{Pi} (%)		FE_{Na} (%)		P_{Pi} (mM)		MAP (mmHg)	
	C	E	C	E	C	E	C	E	C	E
Time Control (n=8)	3.1±0.6	3.6±0.5	21±4	26±4	0.15±0.05	0.13±0.04	1.32±0.05	1.49±0.07	141±5	131±5 *
FGF-23 Full Length (n=8)	3.5±0.3	3.3±0.5	14±3	32±5 *	0.26±0.14	0.76±0.15 *	1.53±0.08	1.49±0.10	146±4	143±5
FGF-23 176-251 (n=8)	3.5±0.5	3.1±0.4	15±2	33±2 *	0.87±0.15	1.21±0.17 *	1.91±0.13	1.91±0.14	117±8	122±8
FGF-23 180-251 (n=8)	4.5±0.4	4.2±0.4	14±3	26±3 *	0.17±0.07	0.76±0.17 *	1.51±0.10	1.42±0.09	150±4	150±5
FGF-23 184-251 (n=8)	4.1±0.3	4.3±0.3	16±3	28±3 *	0.27±0.09	0.63±0.16 *	1.58±0.07	1.46±0.06 *	133±6	128±5
FGF-23 206-251 (n=6)	4.0±0.2	3.1±0.2	22±5	29±2	0.19±0.04	0.22±0.05	1.34±0.06	1.46±0.06	144±4	126±4 *
FGF-23 180-205 (n=6)	4.0±0.5	4.5±0.3	10±2	20±2 *	0.39±0.11	1.01±0.21 *	1.52±0.09	1.54±0.07	151±10	145±7

C = Control Clearance

E = Experimental Clearance

GFR = Glomerular Filtration Rate

FE_{Pi} = Fractional Excretion of Phosphate

FE_{Na} = Fractional Excretion of Sodium

P_{Pi} = Plasma Phosphate Concentration

MAP = Mean Arterial Pressure.

* Indicates a significant difference ($p<0.05$ paired T test), Data are expressed as mean ± SE