Neither Absence Nor Excess of FGF23 Disturbs Murine Fetal-Placental Phosphorus Homeostasis or Prenatal Skeletal Development and Mineralization

Yue Ma,* Manoharee Samaraweera,* Sandra Cooke-Hubley, Beth J. Kirby, Andrew C. Karaplis, Beate Lanske, and Christopher S. Kovacs

Faculty of Medicine–Endocrinology (Y.M., M.S., S.C.-H., B.J.K., C.S.K.), Memorial University of Newfoundland, St John’s, Newfoundland and Labrador, Canada A1B 3V6; McGill University and Jewish General Hospital, Montréal (A.C.K.), Québec, Canada H3T 1E2; and Department of Oral Medicine, Infection and Immunity (B.L.), Harvard School of Dental Medicine, Boston, Massachusetts 02115-5819

Fibroblast growth factor-23 (FGF23) controls serum phosphorus largely through actions on the kidneys to excrete phosphorus and reduce calcitriol. Although these actions are well established in adults and children, the role that FGF23 plays in regulating fetal phosphorus metabolism has not been previously studied. We used several mouse models to study the effect of endogenous deficiency or excess of FGF23 on fetal phosphorus metabolism. We found that intact FGF23 does not cross the placenta from mother to fetus, but wild-type fetuses normally have intact FGF23 levels that approximately equal the maternal level. Deletion of Fgf23 or 7.8-fold higher serum FGF23 levels did not disturb any parameter of fetal mineral homeostasis, including serum and amniotic fluid phosphorus, skeletal morphology, skeletal mineral content, and placental phosphorus transport. Placentas and fetal kidneys abundantly express FGF23 target genes. Cyp24a1 was significantly reduced in Fgf23 null kidneys and was significantly increased in Phex null placentas and fetal kidneys. Phex null kidneys also showed reduced expression of Klotho. However, these changes in gene expression did not disturb any physiological parameter related to phosphorus. A 50% reduction in FGF23 also failed to affect renal phosphorus excretion into amniotic fluid when either PTH or the vitamin D receptor were absent. In conclusion, FGF23 is not an important regulator of fetal phosphorous metabolism. The active delivery of phosphorus across the placenta does not require FGF23, and that process overrides any effects that absence or excess of FGF23 might otherwise have on phosphate handling by the fetal kidneys. (Endocrinology 155: 1596–1605, 2014)

In adults, fibroblast growth factor-23 (FGF23) is a phosphorus-regulating hormone produced largely by osteocytes and osteoblasts (1). It controls the supply of phosphorus at the mineralizing surface of bone through actions on distal tissues. Within proximal renal tubules, FGF23 down-regulates expression of sodium-phosphate cotransporter 2a (NaPi2a) and NaPi2c (2). FGF23 also inhibits the renal 1α-hydroxylase (Cyp27b1), increases expression of 24-hydroxylase (Cyp24a1), and inhibits intestinal expression of NaPi2b (2). The combined effects of these actions lead to increased renal phosphorus excretion and reduced active intestinal absorption of calcium and phosphorus. High serum levels of phosphorus and calcitriol are considered the most potent stimuli for FGF23 synthesis and release (3, 4). PTH stimulates FGF23, whereas FGF23 in turn inhibits PTH (3, 4).

Loss of FGF23 causes hyperphosphatemia, extrskeletal calcifications, and early mortality, which occur as a result of impaired urine phosphorus excretion, increased calcitriol, and increased intestinal phosphorus absorption (5–7). Although PTH also lowers serum phosphate, it is unable to correct the hyperphosphatemia caused by ab...
sence of FGF23. Excess FGF23 leads to the opposite phenotype of hypophosphatemia with impaired mineralization (rickets or osteomalacia) and myopathy, which occur as a consequence of excess renal phosphorus excretion, reduced calcitriol, normal to low PTH, and reduced intestinal phosphorus absorption (5, 7, 8). In X-linked hypophosphatemic rickets, loss of function mutations in the Phex gene cause high circulating levels of FGF23 (9).

The findings described in the preceding paragraphs pertain to study of young and adult humans and mice, whereas phosphorus metabolism during fetal development differs from these developmental periods in several respects. Normal human fetuses are hyperphosphatemic, with cord blood phosphorus being approximately 0.5 mmol/L higher than the maternal value; murine levels are similar (10). The fetal demand for phosphorus is met by active transport across the placenta, which may also contribute to the high serum phosphorus levels. Phosphorus plays key roles during endochondral bone development by inducing apoptosis of hypertrophic chondrocytes (11, 12) and by being incorporated first into osteoid before calcium binds to it (11, 13, 14). Consequently, disturbances in serum phosphorus may alter the formation and mineralization of bone during fetal development. PTH and PTH-related protein (PTHrP) both contribute to fetal phosphorus regulation, because we have previously shown that absence of parathyroids, PTH, or PTHrP each lead to a more than 20% increase in serum phosphorus above the wild-type (WT) fetal value (15–18). Calcitriol may not be an important regulator of fetal phosphorus metabolism, because serum phosphorus was normal in fetuses that lacked the vitamin D receptor (VDR) (19). FGF23 levels were not measured in any of these models.

Until now, little has been known about FGF23’s possible contributions to fetal phosphorus regulation or the expression of its target genes in placenta and fetal kidneys. FGF23 is predominantly expressed in fetal rat osteoblasts, as well as in thymus, liver, and kidney (20). Murine fetuses express FGF23 as early as embryonic day (ED)12.5 in heart, liver, and somites; and later in bone (21). Whether the placenta expresses FGF23 was not examined in those rodent studies. In WT mice, FGF23 levels were 10-fold higher than adult values within 12 hours after birth (22). In human cord blood, C-terminal FGF23 levels were 2-fold adult values, whereas intact FGF23 was 0.2- to 0.3-fold adult values; these divergent results may indicate that FGF23 is abundantly produced but rapidly cleaved in utero (23, 24). Cord blood Klotho was about 6-fold adult and neonatal values (24). Human trophoblasts were shown to express Klotho (24), but placental expression of FGF23 has not been reported.

In this study, we hypothesized that FGF23 regulates fetal phosphorus metabolism through actions on placenta, kidneys, and intestines. To test this, we determined the expression of FGF23 and its target genes in placenta and fetal kidneys, we studied the effect of loss of PTH (Pth null fetuses) or the VDR (Vdr null fetuses) on FGF23 and renal phosphorus excretion, and we compared the effects of loss of FGF23 (in Fg23 null fetuses) with excess FGF23 (in Phex null male fetuses, also called Hyp mice).

Materials and Methods

Animal husbandry

The engineering of Pth, Vdr, and Fgf23 gene deletion models have been previously described (21, 25, 26). C57BL/6-Phex<sup>Hyp-zf</sup>/J (Phex) mice were purchased from The Jackson Laboratory, whereas Vdr mice were a previous gift from Dr Marie Demay (Massachusetts General Hospital). Pth and Vdr mice had been backcrossed into Black Swiss (Taconic) for at least 10 generations before these studies, whereas Fg23 and Phex mice were studied in their original C57BL/6 strains. Each colony was maintained by breeding heterozygous-deleted mice together. Genotyping of Pth, Vdr, and Fgf23 fetuses was done by PCR on DNA extracted from tail clips using previously described primer sequences (17, 19, 27). For the Phex colony, which bears a spontaneous X-linked mutation, several steps were required. Male and female fetuses were first distinguished by PCR to detect the Y-chromosome gene Sry, with B-actin used as a positive control (28). Male mice were separated into WT and Pth null by PCR to detect exon 15 (absent in Phex null) and exon 11 (present in WT and Phex null) (29). The Phex gene has not been sequenced adjacent to exon 15 to enable conventional PCR genotyping of female mice. Instead, RNA extracted from the tail was first reverse transcribed to create a cDNA, and then conventional PCR was used to amplify the Phex sequence. WT females had a single band, whereas Phex<sup>-/-</sup> females had 2 bands, including a shorter mutant band. The specific primers were as follows: Phex-cDNA forward, GGCAACGTACTGCAAACCCGC and Phex-cDNA reverse, CCACAGCACCCAGGTACAGG.

Mice were mated overnight. The presence of a vaginal mucus plug on the morning after mating marked ED0.5. Normal gestation is 19 days. All analyses were done at ED18.5 unless otherwise specified. Adult mice were given a standard chow (1% calcium, 0.75% phosphorus) diet and water ad libitum. The Institutional Animal Care Committee of Memorial University of Newfoundland approved all procedures involving live animals.

Chemical and hormone assays

Serum and amniotic fluid were collected using methods previously described (15, 30). Calcium, phosphorus, and magnesium were analyzed using colorimetric assays (Sekisui Diagnostics PEI, Inc). ELISAs were used to measure PTH 1–34 (Immutoptics), calcitriol (Immunodiagnostic Systems Ltd) and intact FGF23 (Kainos). Any values that appeared to be below the assay sensitivity were reset to values that equaled the respective assay’s detection limit.
Placental phosphorus transfer

We adapted our placental calcium transport methodology (30). Briefly, on ED17.5, pregnant dams were given an intracardiac injection of 50-μCi 32P and 50-μCi 51Cr-EDTA (EDTA is passively transferred and serves as a blood diffusional marker). Five minutes later, the fetuses were removed and later solubilized. The 32P and 51Cr activity within each fetus was separately measured using a liquid scintillation counter and a γ-counter, respectively. Each fetal 32P/51Cr value was normalized within its litter to the mean heterozygous value (in Fgf23 litters) or the mean Phex male null value (in Phex litters) in order that the aggregate results from different litters could be analyzed. The 32P activity alone was also analyzed after normalizing.

Fetal ash and skeletal mineral assay

As previously described (16), intact fetuses (ED17.5 or ED18.5) were reduced to ash in a furnace (500°C × 24 h). A PerkinElmer 2380 Atomic Absorption Flame Spectrophotometer assayed the calcium, phosphorus, and magnesium content of the ash.

Histology

Undecalcified fetal tibias were fixed in 10% buffered formalin, dehydrated in graded alcohol series, and embedded in paraffin. von Kossa staining was performed on 5-μm deparaffinized sections using 1% aqueous silver nitrate solution and 45 minutes of exposure to bright light. The counter stain was 2% methyl green.

RNA extraction and real-time quantitative RT-PCR (qPCR)

Fetal kidneys and placentas were collected at ED18.5 and then snap frozen in liquid nitrogen. Total RNA was purified using the RNasy Midi Lipid kit (QIAGEN). RNA quantity and quality was confirmed with the Agilent 2100 BioAnalyzer (Agilent Technologies). We used TaqMan Gene Expression Assays (with the manufacturer’s predesigned primers and probes for optimal amplification), and Fast Advanced Master Mix from Applied BioSystems (ABI)/Life Technologies, to determine expression of Cyp27b1 (1α-hydroxylase), Cp24a1 (24-hydroxylase), Fgf23, Klotho, and FGF receptors 1 through 4 (Fgfr1–Fgfr4). Details of conditions and cycle times have been previously reported (17, 31, 32). Briefly, cDNA was synthesized using the TaqMan High Capacity cDNA Reverse Transcription kit (ABI), and singleplex qPCRs were run in triplicate on the ViiA 7 Real-Time PCR System (ABI) (17, 19). The minimum sample size was 5 for each genotype (WT and null). Relative expression was determined from the threshold cycle (Ct) normalized to the reference gene (Gapdh).

Statistical analysis

Data were analyzed using StatPlus:Mac Professional 2009, Build 5.8.3.8 (AnalystSoft, Inc). ANOVA was used for analysis of biochemical, transport, and ash data, with a post hoc test to determine which pairs of means differed significantly. qPCR data were analyzed by the comparative Ct method (ΔΔCt) (33). Two-tailed probabilities are reported as mean ± SD.

Results

Pth null vs Vdr null fetuses

PTH stimulates FGF23, and so loss of PTH should reduce FGF23. We previously reported that Pth null fetuses were hyperphosphatemic compared with their WT siblings (3.65 ± 0.2 vs 2.95 ± 0.15 mmol/L) (17). To determine whether reduced FGF23 may contribute to the hyperphosphatemia, we measured intact FGF23 in fetal serum and the content of phosphorus in amniotic fluid (which largely consists of fetal urine). FGF23 was reduced 50% in Pth null fetuses compared with their WT littermates and simultaneous maternal values (Figure 1A). However, amniotic fluid phosphorus was not different between Pth null and WT (Figure 1B).

Calcitriol is a potent stimulator of FGF23 synthesis, and so loss of calcitriol’s actions should also lower se-
We previously reported that Vdr null fetuses have normal serum phosphorus and amniotic fluid phosphorus (19). We found that FGF23 was reduced by more than 50% in Vdr null fetuses compared with their WT siblings and maternal values (Figure 1C). Amniotic fluid phosphorus was not different between Vdr null and WT in new samples (Figure 1D) nor when these data were pooled with the previously published results (data not shown).

Taken together, these findings indicate that PTH and calcitriol are both important determinants of serum FGF23 in WT fetuses and that the FGF23 level in WT fetuses approximately equals the maternal value. However, because a 50% reduction in FGF23 did not disturb serum phosphorus in Vdr null fetuses, it is unlikely that a similar reduction in FGF23 contributed to the hyperphosphatemia in Pth null fetuses. We directly examined the effect of complete absence of FGF23 in the next series of experiments.

Fgf23 null fetuses

Loss of FGF23 leads to marked hyperphosphatemia in young and adult mice (21), and so we examined Fgf23 null fetuses to determine whether loss of FGF23 disturbs fetal phosphorus regulation. We found that intact FGF23 was undetectable in Fgf23 null fetuses, a finding that also confirms that FGF23 does not cross the placenta from the mother (Figure 2A). The FGF23 level in WT fetuses was approximately 25% of the maternal value, unlike what we observed in WT fetuses from the Pth and Vdr colonies.

Despite the absence of FGF23, serum phosphorus and calcium, and amniotic fluid phosphorus and calcium, were not different between Fgf23 null and WT (Figure 2, B–E). Serum PTH was nonsignificantly re-

---

**Figure 2.** Loss of FGF23 does not disturb fetal phosphorus and calcium parameters. Fgf23 null fetuses had undetectable serum FGF23 (A) but normal serum phosphorus (B), calcium (C), and magnesium (data not shown) and normal amniotic fluid phosphorus (D), calcium (E), and magnesium (data not shown). Serum PTH showed a nonsignificant reduction in Fgf23 null fetuses (F). Calcitriol showed the normal finding of being 50% or lower than the maternal value but was not significantly different among the fetal genotypes (G). There was no difference in 32p transport at 5 minutes when corrected for 51Cr-EDTA diffusion (H) or when 32p was analyzed alone (data not shown). Dam+/− indicates that the mothers had to be heterozygous in order to generate Fgf23 null fetuses. The numbers of observations are indicated in parentheses.
duced in Fgf23 nulls (Figure 2F). Fetal calcitriol was less than 50% of the maternal value as we have previously observed in normal fetal mice, and not different between Fgf23 null and WT (Figure 2G). We measured the activity of 32P/51Cr or 32P alone transported from mother to fetus (Figure 2B) as well as ash content of phosphorus (C), calcium (D), and magnesium (data not shown). The ash weight and ash mineral content was also normal at ED17.5 (data not shown). The numbers of observations are indicated in parentheses.

Table 1. Fgf23 and FGF23 Target Gene Expression in Placentas and Fetal Kidneys from WT and Fgf23 null Fetuses

<table>
<thead>
<tr>
<th>Gene</th>
<th>Placenta</th>
<th>Fetal kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>Fgf23 null</td>
</tr>
<tr>
<td>Fgf23</td>
<td>1.00 ± 0.72</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Klotho</td>
<td>1.00 ± 0.16</td>
<td>1.11 ± 0.21</td>
</tr>
<tr>
<td>NaPi2a</td>
<td>1.00 ± 0.13</td>
<td>1.03 ± 0.37</td>
</tr>
<tr>
<td>NaPi2b</td>
<td>1.00 ± 0.40</td>
<td>1.05 ± 0.35</td>
</tr>
<tr>
<td>NaPi2c</td>
<td>1.00 ± 0.41</td>
<td>0.93 ± 0.24</td>
</tr>
<tr>
<td>Cyp24a1</td>
<td>1.00 ± 0.95</td>
<td>1.14 ± 0.22</td>
</tr>
<tr>
<td>Cyp27b1</td>
<td>1.00 ± 0.15</td>
<td>1.18 ± 0.46</td>
</tr>
<tr>
<td>Fgfr1</td>
<td>1.00 ± 0.06</td>
<td>1.21 ± 0.30</td>
</tr>
<tr>
<td>Fgfr2</td>
<td>1.00 ± 0.14</td>
<td>0.96 ± 0.10</td>
</tr>
<tr>
<td>Fgfr3</td>
<td>1.00 ± 0.38</td>
<td>0.76 ± 0.12</td>
</tr>
<tr>
<td>Fgfr4</td>
<td>1.00 ± 0.17</td>
<td>1.00 ± 0.20</td>
</tr>
</tbody>
</table>

Abbreviation: N/A, not applicable.

Parameters that showed statistically significant differences between the 2 genotypes are shown in bold text.
1. We next examined the effect of excess FGF23 in the following experiments.

**Phex null (Hyp) fetuses**

Excess FGF23 leads to marked hypophosphatemia and rickets/osseous dysplasia in children and adults (5, 7, 8, 34). We chose Phex null male fetuses, which recapitulate the human condition X-linked hypophosphatemic rickets, to determine whether excess FGF23 disturbs fetal phosphorus regulation. Similarly, high levels of serum FGF23 were found among Phex null male fetuses, Phex+/− female fetuses, and their Phex+/− mothers; these values were 7.8-fold the value of WT fetuses (Figure 4A). These findings provide additional evidence that FGF23 does not cross the placenta and reveal that mutation of one allele of the X-linked Phex equally increases serum FGF23 in male and female fetuses and their mothers, despite the presence of a normal Phex allele in the females.

Despite increased serum FGF23 within Phex null male fetuses, and a low maternal phosphorus level, Phex null male fetuses had normal serum phosphorus (B) and calcium (C), and magnesium (data not shown) and normal amniotic fluid phosphorus (D), calcium (E), and magnesium (data not shown). Serum PTH was unchanged in Phex null male fetuses compared with WT, although all fetal PTH values were higher than expected for fetal values (F). Calcitriol showed the normal finding of being 50% or lower than the maternal value but was significantly lower in Phex null male fetuses compared with WT (G). There was no difference in 32P transport at 5 minutes when corrected for 51Cr-EDTA diffusion (H) or when 32P was analyzed alone (data not shown). Dam+/− indicates that the mothers had to be heterozygous in order to generate Phex null male fetuses. The numbers of observations are indicated in parentheses.

![Figure 4](https://academic.oup.com/endo/article-abstract/155/5/1596/2423083)
normal lengths, and microscopic assessment of the tibias showed normal morphology and mineralization pattern (Figure 5A). There were no differences in skeletal ash weight, phosphorus, calcium, or magnesium content among the different genotypes (Figure 5B–D).

To determine whether hypophosphatemia in Phex−/− mothers affected fetal serum phosphorus, we also mated WT females to Phex null males. Fetal serum phosphorus levels did not differ between fetal genotypes or by maternal genotype (compare Figure 4B with Supplemental Figure 3).

Phex null male kidneys and placentas both showed a significant increase in Cyp24a1 expression, whereas fetal kidneys also showed small but statistically significant reductions in the expression of Klotho and Fgfr2 and near-significant reductions in Fgfr1, Fgfr4, and NaPi2b (Table 2). Phex null placentas showed an increase in Fgfr3 expression that approached statistical significance (P = 0.054). There was no significant difference between WT and Phex null male placentas or fetal kidneys in the expression of any other FGF23 target gene (Table 2).

**Discussion**

In children and adults, FGF23 regulates serum phosphorous mainly by increasing renal phosphorous excretion and reducing calcitriol (decreased synthesis and increased catabolism). Much of the data examining the role of FGF23 has come from extreme situations, in which FGF23 is either absent or its effective circulating levels are markedly increased. In these extremes, the regulation of phosphorus is disturbed in humans and the corresponding mouse models. Until now, little has been known about the expression of FGF23 and its target genes in placenta or fetal kidneys, or whether FGF23 plays a significant role in regulating fetal phosphorus.

In the present study, we have confirmed that FGF23 target genes are well expressed in fetal kidneys and placentas.
centa. We have previously reported that maternal intact FGF23 doubles during pregnancy compared with prepregnant values (35), and we have now found that intact FGF23 normally circulates in fetal blood at the same level as in the mother. Fgf23 null fetuses had undetectable FGF23, which confirms that intact FGF23 cannot cross the placenta from mother to fetus. This conclusion is also supported by the 7.8-fold higher FGF23 levels in Phex<sup>+/−</sup> mothers that did not appreciably raise the intact FGF23 levels of their WT offspring.

Loss of PTH or VDR each caused at least a 50% reduction in serum intact FGF23, which confirms that PTH and calcitriol are both important stimulators of FGF23 during fetal development. Because marked hyperphosphatemia occurred in Pth null fetuses but serum phosphorus was normal in Vdr null fetuses, this suggests that the reduction in serum FGF23 does not contribute to the hyperphosphatemia of Pth null fetuses. Amniotic fluid phosphorus excretion was not affected in Pth null or Vdr null fetuses, which confirms that the hyperphosphatemia in Pth null fetuses must not be due to reduced renal phosphorus excretion. Pth null fetuses have reduced mineralization of the fetal skeleton (17), whereas Vdr null fetuses have normal skeletal mineralization (19). Therefore, hyperphosphatemia in Pth nulls may result from reduced incorporation of phosphorus into the developing skeleton and not from loss of any effect of FGF23 to regulate phosphate excretion by fetal kidneys.

We more rigorously tested FGF23’s potential role in fetal phosphorus regulation by comparing Fgf23 null and WT fetuses and placentas. We found that FGF23’s known target genes were abundantly expressed in WT fetal kidneys and placenta, which means that the mechanisms are in place for FGF23 to affect fetal phosphorus regulation. However, we found that loss of FGF23 did not significantly alter any measured parameter of fetal mineral homeostasis, including serum and amniotic fluid phosphorus and calcium, placental phosphorus transport, skeletal morphology, or skeletal ash weight and mineral content. Given FGF23’s ability to inhibit PTH, and that loss of FGF23 causes hyperphosphatemia in the adult, we anticipated that Fgf23 null fetuses would show a compensatory increase in serum PTH. However, serum PTH levels were not significantly altered but showed a trend for lower (not higher) PTH values in Fgf23 nulls. Calcitriol was also no different between WT and Fgf23 null.

Loss of Fgf23 caused significantly reduced expression of Cyp24a1 in fetal kidneys, but none of the other FGF23 target genes showed differential expression between WT and Fgf23 null placentas or kidneys. Thus, fetal kidneys did respond to loss of FGF23 by altering expression of 1 target gene, but the relevant physiological responses (renal phosphate handling, serum phosphorus, and serum calcitriol) were unaltered. However, we cannot exclude that absence of FGF23 was compensated by other factors that may act upon the Klotho-FGF receptor complex.

We next studied Phex null fetuses to determine whether excess FGF23 induces hypophosphatemia and renal phosphate wasting as it does in children and adults. However, we found no disturbance in serum and amniotic fluid phosphorus and calcium, placental phosphorus transport, skeletal morphology, or skeletal ash weight and mineral content. WT, Phex null male, and Phex<sup>+/−</sup> female fetuses were able to maintain normal phosphorus parameters despite marked hypophosphatemia in their mothers. Excess FGF23 inhibits PTH, and yet serum PTH levels were not different between WT male and Phex null male fetuses. Increased FGF23 resulted in an increase in Cyp24a1 expression in fetal kidneys and placentas, which is the opposite of the change observed in Fgf23 null kidneys. This may explain the small but statistically significant reduction in serum calcitriol in Phex null male fetuses. Expression of Klotho and Fgfr2 were reduced in Phex null kidneys, but there were no significant changes in the expression of the remaining FGF23 target genes in placentas or kidneys. Fgfr2 expression was reduced only 17% in the Phex null kidneys, and so this may not be a physiologically significant difference. Overall, fetal kidneys responded to high FGF23 levels as they do in the adult by increasing Cyp24a1, decreasing expression of Klotho, and reducing serum calcitriol. However, these changes did not cause any detectable disturbance in renal phosphate handling or serum phosphorus. Because loss of VDR does not disturb fetal phosphorus regulation as we have shown in this article, it is not surprising that modest lowering of calcitriol in Phex null male fetuses had no effect.

We have primarily contrasted the different fetal genotypes to show that loss or excess of FGF23 does not disturb fetal serum phosphorus, amniotic fluid phosphorus, placental phosphorus transport, and skeletal phosphorus content. We also found that neither the maternal serum phosphorus nor the maternal genotype (WT, Fgf23<sup>+/−</sup>, or Phex<sup>+/−</sup>) affected any of those phosphorus parameters. It is possible that the mother’s heterozygous phenotype may influence the fetal phenotype. Although WT fetuses normally have intact FGF23 levels that are equivalent to the maternal level (Figure 1), the serum FGF23 was significantly reduced to less than 50% of the maternal level in WT fetuses from Fgf23<sup>+/−</sup> mothers. Because FGF23 was also reduced in WT fetuses obtained from WT mothers mated to Fgf23<sup>+/−</sup> male mice, the lower FGF23 value may be influenced by the genetic background (C57BL/6 in Fgf23 and Phex mice, and Black Swiss in Pth and Vdr mice). This modest reduction in FGF23 did not otherwise
affect the phenotype of WT fetuses when compared with their Fgf23 null littermates, or when compared with WT fetuses from Phex\(^{+/−}\) mothers.

The maternal phenotype may also be affecting fetal PTH, which normally circulates at low values in the fetal circulation but was higher than expected in fetuses from Phex\(^{+/−}\) mothers. Maternal hypophosphatemia may require fetuses to up-regulate the mechanisms responsible for placental phosphorus transport in order to obtain the needed amount of phosphorus. Conceivably, secondary hyperparathyroidism may stimulate placental phosphate transport. However, studies of perfused placentas from fetal lambs found that exogenous PTH or PTHrP did not stimulate this process (36).

We did not comprehensively examine maternal physiology during pregnancy in these studies. However, we note that 7-fold increased FGF23 in Phex\(^{+/−}\) mothers did not suppress maternal serum calcitriol during pregnancy. The failure of calcitriol to suppress is likely not due to contributions from the placentas and other fetal sources. Calcitriol normally increases 2- to 5-fold during pregnancy, primarily as a result of increased production within maternal kidneys and without the need for stimulation by PTH (35, 37). The placentas normally contribute little if any calcitriol to the maternal circulation, as shown by an anephric woman who had low calcitriol before and during a pregnancy (38) and by 30-fold higher expression of Cyp27b1 in maternal kidneys vs placentas in mice (35).

Why does loss or excess of FGF23 fail to significantly alter fetal renal phosphorus handling, serum phosphorus, or skeletal phosphorus content? Active transport of phosphorus across the placenta is the main route of phosphorus delivery to the fetus. The fetal kidneys and intestines provide only a route for recycling of mineral that comes across the placenta, is excreted by the kidneys into the amniotic fluid, and is then swallowed and reabsorbed. This is evidently a trivial route compared with the substantial forward flux of phosphorus across the placenta. Furthermore, FGF23 is evidently not a major regulator of active placental transport of phosphorus. Absence or excess of FGF23 alters expression of some FGF23 target genes in fetal kidneys in a predictable way, but these changes are in a minor circuit with respect to the placenta and fetal phosphorus regulation; consequently, they do not alter the fetal phenotype.

In summary, intact FGF23 does not cross the placenta from mother to fetus. Deletion of Fgf23 or 7.8-fold higher serum FGF23 did not disturb any measured parameter of fetal mineral homeostasis, including serum and amniotic fluid phosphorus, skeletal morphology, skeletal mineral content, and placental phosphorus transport. WT placentas display low level expression of Fgf23, which is absent in Fgf23 null placentas and modestly increased in Phex null male placentas. Placentas and kidneys abundantly express FGF23 target genes, but Cyp24a1 was the only gene showing altered expression in Fgf23 null kidneys. Excess FGF23 in Phex null fetuses increased the expression of Cyp24a1 in fetal kidneys and placentas and reduced the expression of Klotho in fetal kidneys only. These changes in gene expression did not disturb any measured physiological parameter related to phosphorus. A 50% reduction in FGF23 also failed to affect renal phosphorus excretion into amniotic fluid when PTH or VDR were also absent.

In conclusion, despite FGF23’s presence in the fetal circulation and expression of its target genes in placenta and kidneys, FGF23 is not an important regulator of fetal phosphorus. Phosphorus is actively delivered across the placenta through means that do not require FGF23, and this process may override any effects that absence or excess of FGF23 might otherwise have on fetal kidneys. At birth, the placental phosphorus pump is lost, and that is when FGF23 gains importance as a regulator of renal phosphorus excretion and intestinal phosphorus absorption.

Acknowledgments

Medical student Sri Teja Vatturi and medical resident Subramanian Suppiah contributed to the collection of fetal samples during their respective research electives. Selected results have been presented in abstract form at the second Joint Meeting of the International Bone and Mineral Society and the Japanese Society for Bone and Mineral Research, the American Society for Bone and Mineral Research, and the European Calcified Tissue Society.

Address all correspondence and requests for reprints to: Dr Christopher S. Kovacs, Health Sciences Centre, 300 Prince Philip Drive, St. John’s, Newfoundland and Labrador, Canada A1B 3V6. E-mail: ckovacs@mun.ca.

This work was supported by Canadian Institutes of Health Research Grants 84253 and 126469 and the Research and Development Corporation of Newfoundland Grant 5404.1145.102 (to C.S.K.) and by the National Institutes of Health Grant DK097105 (to B.L.).

Disclosure Summary: The authors have nothing to disclose.

References

3. Yuan Q, Sato T, Densmore M, et al. FGF-23/Klotho signaling is not
essential for the phosphaturic and anabolic functions of PTH. J Bone Miner Res. 2011;26(9):2026–2035.