FGF23 and Klotho in Bone Mineralization in CKD

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Objectives

• 1. To understand the direct effects of FGF23 on mineral metabolism

• 2. To describe changes in FGF23 and Klotho levels in CKD

• 3. To understand complexities of the studies evaluating the direct effect of FGF23 and Klotho on bone in human CKD
FGF23: what it does

Is there a direct effect of FGF23 on bone?

Too much: hypophosphatemic rickets

Too little: tumoral calcinosis
FGF23: where it’s made

**Bone**
Correlation with blood levels (anuric):
r=0.7; p<0.01
Pereira R et al Bone 2009

**Cirrhotic liver**
Wasserman H et al Pediatrics 2016

**Diabetic kidney**
Zanchi C et al PLOSOne 2013

**Hypertrophied heart muscle**
Leifheit-Nestler M et al Moll Cell Pediatr 2014
Klotho is expressed in osteocytes; Klotho null mice have osteoporosis

Wild type  Klotho-/-

Rhee Y et al Bone 2011

Kuro-o M et al Nature 1997
Mineralization defects co-exist with increased FGF23 in early CKD

When you take away the phosphaturia, could FGF23 be beneficial to mineralization?

Bone turnover

Mineralization

Bone FGF23 (osteocytes/Bone Area)

- OS/BS: \( r = -0.60, p<0.01 \)
- OV/BV: \( r = -0.60, p<0.01 \)
- O.Th: \( r = -0.43, p<0.05 \)

Pereira R et al Bone 2009

Wesseling-Perry K et al CJASN 2013

Isakova T et al Kidney Int 2009
Direct effects of FGF23 and Klotho: confounding effects of 1,25D

Andrukhova O et al JBMR 2017
1,25D directly inhibits bone mineralization

Lieben L et al J Clin Invest 2012
FGF23 null fetuses have normal phosphate and skeletons

Developmental change in receptors or a sign of very little effect on bone?

Ma Y et al Endocrinology 2017
Klotho null fetuses also have normal skeletons

Developmental change in receptors or a sign of very little effect on bone?
Forced FGF23 over-expression suppresses osteoblast differentiation and mineralization

Wang H et al JBMR 2008
Osteocyte-specific Klotho deletion increases bone formation and mass

Table 1 | Serum and urine biochemistry of 5-week-old control mice and Dmp1-Klotho⁻/⁻ mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dmp1-Klotho⁻/⁻</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.4 ± 0.4</td>
<td>9.5 ± 0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Phosphate (mg/dl)</td>
<td>9.7 ± 1.4</td>
<td>9.4 ± 0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>1,25(OH)₂D (pg/ml)</td>
<td>143 ± 55</td>
<td>173 ± 37</td>
<td>0.3</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>211 ± 116</td>
<td>188 ± 64</td>
<td>0.7</td>
</tr>
<tr>
<td>Intact FGF23 (pg/ml)</td>
<td>234 ± 82</td>
<td>245 ± 103</td>
<td>0.8</td>
</tr>
<tr>
<td>C-terminal FGF23 (pg/ml)</td>
<td>310 ± 89</td>
<td>344 ± 65</td>
<td>0.4</td>
</tr>
<tr>
<td>FEP (%)</td>
<td>31.8 ± 28.3</td>
<td>36.7 ± 28.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Komaba H et al Kidney Int 2017
Overexpression of Klotho inhibits mineralization of MC3T3 cells

Komaba H et al Kidney Int 2017
Exogenous FGF23 and Klotho together inhibit osteoblast mineralization

MC3T3.E1

Shalhoub V et al Calcif Tissue Int 2011
FGF23 and Klotho inhibit osteoblastic Wnt signaling in CKD

Klotho is down-regulated in CKD bone; particularly in high phosphate conditions.

Table 2 | Serum and urine biochemistry of 12-week-old control mice and Dmp1-Klotho<sup>−/−</sup> mice with sham operation or 5/6 nephrectomy (NTx) under normal or high-phosphate diet feeding (HPD)

<table>
<thead>
<tr>
<th></th>
<th>Sham Control</th>
<th>Dmp1-Klotho&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>NTx Control</th>
<th>Dmp1-Klotho&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>NTx + HPD Control</th>
<th>Dmp1-Klotho&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Two-way ANOVA Genotype NTx/HPD Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>10.5 ± 3.2</td>
<td>11.8 ± 2.0</td>
<td>42.0 ± 6.0</td>
<td>33.4 ± 15.6</td>
<td>39.3 ± 3.7</td>
<td>41.0 ± 5.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.4 ± 0.5</td>
<td>9.7 ± 1.4</td>
<td>10.2 ± 0.6</td>
<td>10.0 ± 0.4</td>
<td>10.3 ± 0.4</td>
<td>9.8 ± 0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Phosphate (mg/dl)</td>
<td>9.6 ± 1.4</td>
<td>9.4 ± 1.2</td>
<td>9.5 ± 3.9</td>
<td>8.5 ± 3.6</td>
<td>10.7 ± 1.8</td>
<td>11.1 ± 1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>1,25(OH)&lt;sub&gt;2&lt;/sub&gt;D (pg/ml)</td>
<td>99 ± 30</td>
<td>111 ± 62</td>
<td>151 ± 50</td>
<td>168 ± 50</td>
<td>152 ± 57</td>
<td>219 ± 64</td>
<td>0.06</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>308 ± 207</td>
<td>273 ± 190</td>
<td>685 ± 248</td>
<td>581 ± 450</td>
<td>931 ± 916</td>
<td>812 ± 399</td>
<td>0.5</td>
</tr>
<tr>
<td>Intact FGF23 (pg/ml)</td>
<td>280 ± 112</td>
<td>286 ± 118</td>
<td>580 ± 203</td>
<td>644 ± 237</td>
<td>11850 ± 4302</td>
<td>14863 ± 6565</td>
<td>0.3</td>
</tr>
<tr>
<td>C-terminal FGF23 (pg/ml)</td>
<td>556 ± 209</td>
<td>655 ± 262</td>
<td>1561 ± 552</td>
<td>1820 ± 549</td>
<td>9111 ± 3911</td>
<td>11392 ± 4153</td>
<td>0.2</td>
</tr>
<tr>
<td>FEP (%)</td>
<td>12.4 ± 5.3</td>
<td>15.6 ± 5.4</td>
<td>24.4 ± 6.0</td>
<td>28.9 ± 7.2</td>
<td>42.3 ± 12.2</td>
<td>42.0 ± 14.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Komaba H et al Kidney Int 2017
CKD mimics the effect of Klotho-/-; there is no added effect on bone
Primary osteoblasts from dialysis patients mineralize poorly in culture

Healthy controls

Dialysis patients

Alizarin Red (absorbance)

1 week 2 weeks 3 weeks

Pereira RC et al Kidney Int 2018
... this equates to defective maturation
Phosphate, but not FGF23, plays a role in osteoblast maturation.
In human CKD, FGF23 is a marker of early osteocytes

Pereira RC et al Kidney Int 2018
FGF23-expressing osteocytes characterize early 2° mineralization

Pereira RC et al Kidney Int 2018
Summary

• FGF23 and Klotho both affect phosphate and 1,25D and both are regulated by 1,25D

• Klotho (and maybe FGF23+Klotho) may suppress osteoblast maturation

• While CKD bone disease mimics the effects of Klotho deficiency, primary human osteoblasts in vitro maintain intrinsic defects in maturation

• FGF23 may be a marker of early osteocytes in CKD bone