Disturbances of Wnt/β-catenin pathway and energy metabolism in early CKD: effect of phosphate binders

Rodrigo B. de Oliveira¹, Fabiana G. Graciolli¹, Luciene M. dos Reis¹, Ana L.E. Cancela¹, Lilian Cuppari², Maria E. Canziani², Aluizio B. Carvalho², Vanda Jorgetti¹ and Rosa M.A. Moysés¹

¹Nephrology Department, University of São Paulo (USP), São Paulo, Brazil and
²Division of Nephrology, Federal University of São Paulo (EPM-UNIFESP), São Paulo, Brazil

Correspondence and offprint requests to: Rosa M.A. Moysés; E-mail: rosa.moyses@uol.com.br

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ABSTRACT

Background. Mineral bone disorder (MBD) is an early complication of chronic kidney disease (CKD), with complex interactions in the bone–kidney–energy axis. These events lead to impaired bone remodelling, which in turn is associated with cardiovascular disease. Recently, we reported on a positive effect of phosphate binder treatment on bone remodelling markers and a reduction in serum FGF-23 levels in predialysis-CKD patients. The goal of the present study of this trial was to examine the effects of phosphate binders on energy-regulating hormones and Wnt pathway.

Methods. In this present post hoc analysis of the above randomized, open-label, 8-week trial, which compared the effects of increasing doses of sevelamer-HCl or calcium acetate on various CKD-MBD parameters in 40 normophosphatemic CKD Stage 3–4 patients, we measured serum sclerostin, Dickkopf-1, leptin, adiponectin and serotonin concentrations.

Results. Serum sclerostin, Dickkopf-1 and leptin were elevated at baseline despite normal calcium, phosphorus levels and daily urinary phosphorus excretion. There were significant and positive correlations between sclerostin and FGF-23, as well between leptin and Dickkopf-1. Treatment with both phosphate binders led to a significant decrease in phosphate overload. However, sevelamer-HCl, but not with calcium acetate, led to a significant decrease in serum FGF-23, sclerostin and leptin, and to a significant increase in bone alkaline phosphatase levels.

Conclusions. Early stages of CKD are associated with an impairment of the Wnt pathway, as reflected by elevated sclerostin, and a dysregulation of energy-regulating hormones. Many of these disturbances can be ameliorated by phosphate binder treatment, more with sevelamer-HCl than with calcium acetate.

INTRODUCTION

Mineral bone disorder (MBD) is a common complication of chronic kidney disease (CKD) that includes biochemical abnormalities, soft-tissue calcifications and bone disease [1, 2]. Traditionally, biochemical abnormalities include changes in classical bone metabolism markers, such as calcium, phosphorus, parathyroid hormone (PTH) and vitamin D sterols. However, in early stages of CKD, the majority of patients do
not show alterations in most of these parameters, in contrast to later stages of CKD [3].

Recently, it has become increasingly clear that the MBD is an early event in the course of the CKD. New molecules and pathways involved in this disorder have been described, enhancing our understanding of the bone–kidney axis and its repercussion on the cardiovascular system. In this context, fibroblast growth factor-23 (FGF-23), Wnt/β-catenin signalling and the pathogenetic role of the osteocyte have gained considerable interest [4–6].

The known early increase in serum FGF-23 allows serum phosphorus and PTH levels to stay in the normal range for a prolonged time period in the course of CKD [7]. This mechanism should have beneficial effects at least initially. However, with the progression of the disease a striking rise of serum FGF-23 levels into supraphysiological levels is observed in most of the patients. Some authors consider this increase as a maladaptation syndrome, since several studies have shown that such elevated FGF-23 levels were independently associated with the progression of CKD, direct myocardial toxicity, and poor outcomes [8–12]. On the other hand, complete neutralization of FGF-23 by a specific antibody has been shown to be harmful as well, since it induced hyperphosphatemia, vascular calcification and mortality in a rat model of CKD-MBD [13].

Another early event in the course of CKD-MBD is the repression of osteocyte Wnt/β-catenin signalling [5, 14]. The increase in sclerostin expression, a Wnt antagonist, leads to an increase in RANKL expression and in osteoclast activity [14]. There is also evidence showing that loss of β-catenin signalling in osteocytes is linked with a corresponding decrease in the expression of genes associated with osteoblast function. These events lead to impaired bone remodelling, which in turn may be associated with cardiovascular disease [15–18]. However, the mechanism of sclerostin regulation is not yet fully understood and other regulators of mineral metabolism may modulate sclerostin expression as well.

Beyond the well-known role of CKD in renal osteodystrophy, it has recently been found that the bone is also involved in the regulation of energy metabolism, with complex interactions between serotonin and hormones produced by fat tissue, such as adiponectin and leptin [19, 20]. In line with these observations, several experimental and clinical studies were stored at 20°C and only non-thawed samples were used−20°C and only non-thawed samples were used.

In the context of CKD, this hormone is considered as a uremic toxin [26], and the hypothesis of its deleterious effects on patient outcomes has been explored.

Considering the pluripotent effects of FGF-23, Wnt/β-catenin signalling and molecules related to energy metabolism, the hypothesis of their possible pharmacological modulation was explored by several groups, with the aim to improve bone health and to obtain cardio-protective effects [27–33]. In this context, phosphate binders could be useful. Although they are frequently prescribed in CKD patients, only few studies examined their effects on these non-classical markers of the bone–kidney–energy axis [34, 35].

Recently, our group reported a positive effect of the early prescription of phosphate binders on bone remodelling parameters in normophosphatemic stage 3–4 CKD patients, in addition a reduction in serum FGF-23 levels by sevelamer-HCl, but not by calcium acetate [35]. In the present study, we performed a post hoc analysis of that trial, with the aim to examine the effects of phosphate binders on serum adiponectin, leptin, serotonin, sclerostin and Dickkopf-1 levels, and more generally speaking, to explore possible connections between bone-derived molecules and energy metabolism and the effects of phosphate binders thereon in early stages of CKD.

Subjects and Methods

Study design

This is a post hoc analysis of a randomized clinical trial that has been reported previously [35]. Briefly, 40 adult, clinically stable patients with CKD Stages 3–4 from the CKD outpatient clinic of the EPM-UNIFESP in São Paulo, Brazil were selected and randomized in a 1:1 ratio to receive open-label calcium acetate (PhosLo, 667 mg tablets; Fresenius Medical Care, Waltham, MA) or sevelamer-HCl (Renagel, 800 mg tablets; Genzyme Co., Cambridge, MA). The study was conducted over an 8-week period, which included 6-week titration of the two phosphate binders and 2-week washout period. After randomization, patients received calcium acetate, initially at a 1.32 g/day dose, then doubled every 2 week (2.64 and 5.28 g/day) or sevelamer-HCl, initially at a 1.6 g/day dose, then doubled every 2 weeks (3.2 and 6.4 g/day). The washout period was included as a treatment control because this study did not have a placebo control group. Exclusion criteria were body mass index (BMI) <17 or >37 kg/m², proteinuria >3.5 g/24 h, diabetes mellitus, PTH >500 pg/mL or patients receiving any drug that could interfere with mineral metabolism. Informed consent was obtained from all patients. That study was approved by the local ethics committee and was registered on the Brazilian official trial registry (SISNEP) under the number CAAE-0714.0.015.000-07.

Laboratory measurements

Blood samples were collected at baseline, week 6 (post-treatment) and week 8 (post-washout period). The samples were stored at −20°C and only non-thawed samples were used for all analyses. They were used for measurements of human sclerostin (ELISA, Quidel Corporation, USA; reference range: 0.2–0.6 ng/mL; intra- and interassay coefficients of variation are 3.1 and 3.5%, respectively); Dickkopf-1 (ELISA, Assay Designs, USA; normal values in 12 human samples are 3.1 and 3.5%, respectively); human leptin (ELISA, Assay Designs, USA; normal values in 12 human samples are 3.1 and 3.5%, respectively); human leptin (ELISA, Quidel Corporation, USA; normal values in 967 adults healthy volunteers are between 0.23 and 28 ng/mL; intra- and interassay coefficients of variation are 5.4–6.8 and 6.8–8.3%, respectively); total human adiponectin (Tadip) (ELISA, Quidel Corporation, USA; normal values in 226 adults healthy volunteers are between 2.4 and 19.3 µg/mL; intra- and interassay coefficients of variation are 2.4–4.7 and 5.7–6.7%, respectively); serotonin (ELISA, IBL-America, USA;
reference range: 40–450 ng/mL; intra- and interassay coefficients of variation are 3.9–5.4 and 6.0%, respectively). To note, in the present study, only serum Dickkopf-1 levels were not measured at post-washout period.

**Statistical analyses**

Demographic characteristics and biochemical parameters were described as mean ± standard deviation (SD) [except for FGF-23 and PTH, which were expressed as median (25th–75th percentile)]. Spearman’s rank correlations were used to summarize associations. Changes from baseline [i.e., delta: 6th week values (post-treatment values) minus baseline values] were compared between treatment groups using Wilcoxon rank-sum tests. Changes from baseline were compared within treatment groups with the Wilcoxon signed rank. A two-sided P value < 0.05 was considered as statistically significant. Statistical analyses were performed using the SPSS version 17.0 software.

**RESULTS**

**Baseline studies**

**General biochemistry findings.** Forty patients were randomized to each phosphate binder group (calcium acetate, n = 19; sevelamer-HCl, n = 21) and there were no significant differences between the two groups at baseline (Table 1). Based on estimated glomerular filtration rate by Cockroft-Gault formula (eGFR), 22 patients were classified as having CKD stage 3, and 18 as having CKD stage 4.

Despite normal serum calcium, phosphorus and daily urinary phosphorus excretion, patients had an increased fractional phosphate excretion (59.7 ± 32.4%) and serum PTH levels. All but five patients had elevated serum FGF-23 levels (Table 1). FGF-23 correlated with eGFR (R = −0.443; P = 0.004), PTH (R = 0.313, P = 0.049) and deoxypyridinoline (DPD) (R = 0.390; P = 0.014).

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**Table 1. Baseline demographical, clinical and biochemical parameters of the total study population and the two subgroups, calcium acetate-treated patients and sevelamer-HCl-treated patients**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All</th>
<th>Calcium acetate</th>
<th>Sevelamer-HCl</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>19</td>
<td>21</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 ± 11</td>
<td>51 ± 10</td>
<td>50 ± 13</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.28 ± 4.58</td>
<td>26.95 ± 4.16</td>
<td>25.67 ± 4.95</td>
<td>18–25</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>21/19</td>
<td>9/10</td>
<td>12/9</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>2.55 ± 0.78</td>
<td>2.52 ± 0.76</td>
<td>2.59 ± 0.82</td>
<td>0.6–1.2</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>34.5 ± 15.9</td>
<td>32 ± 9.9</td>
<td>36.9 ± 20</td>
<td>75–125</td>
</tr>
<tr>
<td>Total calcium (mg/dL)</td>
<td>9.3 ± 0.5</td>
<td>9.3 ± 0.6</td>
<td>9.2 ± 0.4</td>
<td>8.6–10.2</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.5 ± 0.6</td>
<td>3.6 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>2.7–4.5</td>
</tr>
<tr>
<td>25OH vitD (ng/mL)</td>
<td>34.7 ± 20.6</td>
<td>35.7 ± 18.7</td>
<td>33.9 ± 22.7</td>
<td>&gt;30</td>
</tr>
<tr>
<td>bAP (U/L)</td>
<td>35.1 ± 11.5</td>
<td>36.9 ± 12.6</td>
<td>33.5 ± 10.3</td>
<td>11.6–42.7</td>
</tr>
<tr>
<td>DPD (nmol/L)</td>
<td>10.3 ± 3.4</td>
<td>9.8 ± 3.2</td>
<td>10.8 ± 3.5</td>
<td>3.25 ± 0.66</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>101 (68–131)</td>
<td>89 (52–141)</td>
<td>107 (76–130)</td>
<td>10–65</td>
</tr>
<tr>
<td>FGF-23 (pg/mL)</td>
<td>97 (63–143)</td>
<td>97 (62–148)</td>
<td>103 (62–142)</td>
<td>8.2–54.3</td>
</tr>
<tr>
<td>Sclerostin (ng/mL)</td>
<td>0.80 ± 0.78</td>
<td>0.84 ± 0.54</td>
<td>0.76 ± 0.96</td>
<td>0.2–0.6</td>
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<td></td>
<td>0.73 (0.24–1.05)</td>
<td>0.78 (0.41–1.06)</td>
<td>0.59 (0.1–1.04)</td>
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<tr>
<td>Dickkopf-1 (ng/mL)</td>
<td>18.2 ± 5.7</td>
<td>17.8 ± 4.8</td>
<td>18.6 ± 6.4</td>
<td>0.3–2.3</td>
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<tr>
<td></td>
<td>17 (14.4–20.9)</td>
<td>17.2 (14.7–20.2)</td>
<td>17 (13.5–21.7)</td>
<td></td>
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<tr>
<td>Leptin (ng/mL)</td>
<td>34.5 ± 43</td>
<td>33.5 ± 33.3</td>
<td>35.3 ± 50.8</td>
<td>0.23–28</td>
</tr>
<tr>
<td></td>
<td>17.7 (5.2–52)</td>
<td>26 (5.7–46.2)</td>
<td>9.8 (5–55.5)</td>
<td></td>
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<tr>
<td>Tadip (µg/mL)</td>
<td>11.9 ± 5.9</td>
<td>11.8 ± 5.2</td>
<td>12 ± 6.7</td>
<td>2.4–19.3</td>
</tr>
<tr>
<td></td>
<td>11 (8–14)</td>
<td>11.2 (7.1–16.1)</td>
<td>10.6 (8.3–12.8)</td>
<td></td>
</tr>
<tr>
<td>Serotonin (ng/mL)</td>
<td>220 ± 102</td>
<td>218 ± 115</td>
<td>222 ± 90</td>
<td>40–450</td>
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<tr>
<td></td>
<td>224 (141–296)</td>
<td>252 (127–325)</td>
<td>223 (143–271)</td>
<td></td>
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</tbody>
</table>

Values expressed as mean ± SD and/or median (25th–75th). BMI, body mass index; eGFR, estimated glomerular filtration rate; 25OHvitD, 25-OH vitamin D; bAP, bone-specific alkaline phosphatase; DPD, deoxypyridinoline; PTH, parathyroid hormone; FGF-23, fibroblast growth factor-23; Tadip, total adiponectin.
Wnt pathway inhibitors findings

Mean (± SD) serum sclerostin levels were elevated (0.80 ± 0.78 ng/mL), with 23 patients (57.5%) having values above the normal range. A positive correlation between serum sclerostin levels and FGF-23 (R = 0.362; P = 0.022) was found. Serum Dickkopf-1 was above the normal range in all patients, with mean levels of 18.2 ± 5.7 ng/mL (Table 1). There was a significant correlation between serum levels of Dickkopf-1 and phosphorus (R = 0.393; P = 0.01), and between serum levels of Dickkopf-1 and bone alkaline phosphatase (bAP) (R = 0.317; P = 0.046).

Energy metabolism hormones findings

Mean serum serotonin and Tadip levels were in the normal range. Mean serum leptin levels were elevated (34.5 ± 43 ng/mL) with 16 patients (40%) having hormone levels above the reference values (Table 1). Serum leptin correlated with BMI (R = 0.607; P = 0.0001), serum albumin (R = −0.391; P = 0.013), Dickkopf-1 (R = 0.471; P = 0.002) and 25OH vitamin D (R = −0.419; P = 0.007). Serum serotonin levels correlated with BMI (R = −0.397; P = 0.01), urinary phosphorus (R = −0.388; P = 0.018) and eGFR (R = −0.429; P = 0.006).

Post-treatment studies

Effects of phosphate binder treatment in all patients. After treatment with both phosphate binders, there was a progressive decline in serum PTH, FGF-23, 25OH vitamin D and urinary phosphorus (490 ± 151 versus 280 ± 131 ng/mL, P = 0.0001) whereas bAP increased, as well as urinary calcium (24.3 ± 25.6 versus 36.3 ± 24.1 mg/24 h, P = 0.001) and eGFR (32.5 ± 9.1 versus 35.9 ± 10.7 mL/min/1.73 m², P = 0.001) (Table 2). We also observed a significant decrease in serum leptin. No changes were observed in serum calcium, phosphorus, calcitriol, DPD, sclerostin, Dickkopf-1, serotonin and Tadip levels, when data from all patients were pooled (Table 2).

Effects of phosphate binder treatment on general biochemistry by patient group. There was a significant decrease in serum FGF-23 only in sevelamer-HCl group, but not in calcium acetate group. In addition, the changes from baseline (delta) were significantly different between the groups (−54 ± 65 in sevelamer-HCl group versus −16 ± 49 pg/mL in calcium acetate group, P = 0.04).

Also in sevelamer-HCl group, there was a greater increase in bAP, although changes from baseline were not significantly different between treatment groups (+5.1 ± 8.8 in sevelamer-HCl group versus +1 ± 5.6 U/L in calcium acetate group, P > 0.05). There also was a significant decrease in 25OH vitamin D in sevelamer-HCl group but the changes from baseline were not significant between treatment groups (−5.2 ± 6.8 ng/mL in sevelamer-HCl group versus −2.4 ± 8.8 ng/mL in calcium acetate group, P > 0.05) (Table 2).

Effects of phosphate binder treatment on Wnt pathway inhibitors by patient group. Taking into account each group after phosphate binder therapy, there was a significant decrease of serum sclerostin levels in sevelamer-HCl group, whereas such an effect was not observed in calcium acetate group. Additionally, the changes from baseline were significantly different between the groups (−0.12 ± 0.3 ng/mL in sevelamer-HCl group versus +0.03 ± 0.2 ng/mL in calcium acetate group, P = 0.02) (Figure 1). No changes were observed in serum Dickkopf-1 levels (Table 2).

Effects of phosphate binder treatment on energy metabolism hormones by patient group. Serum leptin levels were significantly decreased in the sevelamer-HCl group, but not in calcium acetate group. However, there were no differences between treatment groups regarding changes from baseline (−10.5 ± 31.1 ng/mL in sevelamer-HCl group versus −1.4 ± 10.8 ng/mL in calcium acetate group, P > 0.05) (Figure 2). No changes were seen regarding serum Tadip and serotonin levels (Table 2).

After the washout period (week 8), all parameters values measured in this present study were similar to those found at the baseline in both groups (Table 2).

DISCUSSION

Our study shows three main findings. First, many non-classical CKD-MBD markers are altered in the early stages of CKD despite normal serum calcium, phosphorus and daily urinary phosphorus excretion, as shown by elevated serum levels of FGF-23, sclerostin, Dickkopf-1 and leptin. Second, in CKD there may be interrelations between the Wnt pathway and hormones involved in energy metabolism, since we found correlations between serum sclerostin and FGF-23, as well as between serum leptin and Dickkopf-1. These associations are in support of the hypothesis of a bone–kidney–energy axis [20]. Third, phosphate binder treatment improves, at least partially, the altered bone–kidney–energy axis in CKD, with more marked effects of sevelamer-HCl than calcium-containing binders.

In our study, phosphate binder treatment reduced phosphorus overload (evidenced by reduction in fractional phosphate excretion), as well as FGF-23, PTH and leptin levels. However, a more detailed analysis revealed that the reduction in serum FGF-23, leptin and sclerostin levels were observed only in sevelamer-HCl-treated patients, who also presented a significant increase in serum bAP levels. To the best of our knowledge, this is the first report showing that a given phosphate binder can improve both serum leptin and sclerostin levels.

Sclerostin and Dickkopf-1 are inhibitors of Wnt signalling, which leads to a decrease in osteoblast activity and hence bone formation. According to present knowledge glucocorticoids, bone morphogenetic protein and calcitriol stimulate sclerostin expression, whereas intermittent PTH administration decreases it [36, 37]. In our study, serum sclerostin and Dickkopf-1 levels were positively correlated with FGF-23 and phosphorus, respectively. Therefore, we hypothesize that CKD-MBD has an impact on Wnt pathway and that phosphorus and FGF-23 could be additional modulators of sclerostin and Dickkopf-1 expression.
Table 2. Serum biochemical parameters at baseline and 6th week (post-treatment) in patients treated with calcium acetate (n = 19) or sevelamer-HCl (n = 21)

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>All</th>
<th>Calcium acetate group</th>
<th>Sevelamer-HCl group</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 6</td>
<td>Week 8</td>
<td>Baseline</td>
</tr>
<tr>
<td>Total calcium (mg/dL)</td>
<td>9.3 ± 0.5</td>
<td>9.3 ± 0.5</td>
<td>9.3 ± 0.5</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.5 ± 0.6</td>
<td>3.4 ± 0.6</td>
<td>3.6 ± 0.6</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>101 (70–130)</td>
<td>72 (42–102)a</td>
<td>111 (64–136)</td>
<td>89 (52–141)</td>
</tr>
<tr>
<td>bAP (U/L)</td>
<td>35.1 ± 11.5</td>
<td>38.3 ± 15a</td>
<td>–</td>
<td>36.9 ± 12.6</td>
</tr>
<tr>
<td>DPD (nmol/L)</td>
<td>10.3 ± 3.4</td>
<td>9.9 ± 3.3</td>
<td>–</td>
<td>9.8 ± 3.2</td>
</tr>
<tr>
<td>FGF-23 (pg/mL)</td>
<td>97 (64–142)</td>
<td>63 (46–113)a</td>
<td>111 (53–133)</td>
<td>97 (62–148)</td>
</tr>
<tr>
<td>25OH vitD (ng/mL)</td>
<td>34.7 ± 20.6</td>
<td>30.8 ± 17a</td>
<td>–</td>
<td>35.7 ± 18.7</td>
</tr>
<tr>
<td>Sclerostin (ng/mL)</td>
<td>0.80 ± 0.78</td>
<td>0.73 (0.24–1.05)</td>
<td>0.73 ± 0.76</td>
<td>0.73 (0.28–0.94)</td>
</tr>
<tr>
<td>Dickkopf-1 (ng/mL)</td>
<td>18.2 ± 5.7</td>
<td>17 (14.4–20.9)</td>
<td>18.1 ± 6</td>
<td>16.9 (13.6–20.5)</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>34.5 ± 43</td>
<td>17.7 (5.2–52)</td>
<td>27.3 ± 28.5a</td>
<td>14.8 (4.2–47.2)</td>
</tr>
<tr>
<td>Tadip (µg/mL)</td>
<td>11.9 ± 5.9</td>
<td>11 (8–14)</td>
<td>14.6 ± 10.4</td>
<td>10 (7.5–18.5)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD and/or as median (25th–75th percentile interval). 25OHvitD, 25-OH vitamin D; bAP, bone-specific alkaline phosphatase; DPD, deoxypyridinoline; PTH, parathyroid hormone; FGF-23, fibroblast growth factor-23; Tadip, total adiponectin.

Within-group change from baseline; P < 0.05. 
Between-group change; P < 0.05.
In this regard, our group recently showed in CKD rats subjected to parathyreoidectomy to prevent secondary hyperparathyroidism and high-turnover bone disease, that a high phosphate diet was associated with an increase in both serum and bone sclerostin levels, as well as with low bone volume. These findings reinforce the role of phosphorus in sclerostin regulation through a PTH-independent mechanism (Ferreira et al. Regulation of sclerostin expression and bone volume by phosphate in a model of adynamic bone disease, Abstract Title [Abstract]. J Am Soc Nephrol 23: 78A, 2012). However, the reduction in sclerostin observed in our sevelamer-HCl-treated patients cannot be attributed to a different degree of serum phosphorus control, since both patient groups experienced a similar decrease in phosphorus overload, as indicated by similar serum and fractional phosphate excretion at baseline and post-treatment. An alternative explanation is that the increase of calcium load in the calcium acetate group may also contribute to sclerostin elevation. Nevertheless, although we do not know by which mechanisms sevelamer-HCl treatment led to the observed reduction of serum sclerostin, this novel observation opens a window for therapeutic opportunities in CKD patients since there is evidence from animal models of osteoporosis and clinical studies in postmenopausal women that sclerostin antibody administration can improve bone quality [31,38].

In the present post hoc study, we observed that sevelamer-HCl-treated patients experienced a reduction in serum sclerostin together with a reduction in serum PTH and an increase in serum bAP levels. These findings are in favour of improved bone remodelling.

Of note, we observed a remarkable elevation in serum Dickkopf-1 levels taking into account all the patients and both groups separately, as well as in the baseline and week 6. Thambiah et al. [39] also found elevated serum Dickkopf-1 levels, although in a lesser degree than observed in our study. Both studies are in opposition to the normal serum Dickkopf-1 levels observed by Cejka et al. [5]. We propose three main hypotheses to these inconsistencies: first, populations from different CKD stages should have different Dickkopf-1 modulator factors, which possibly lead to different Dickkopf-1 expression. Second, CKD from different etiologies should have various CKD-MBD courses, resulting in many profiles of Wnt/β-catenin disturbances. Third, Dickkopf-1 measurement should be affected by the utilization of distinct assays from different manufacturers.

Regarding leptin, its seems to exert effects on bone in addition to its classical actions [20] including its role in cardiovascular disease [21–25]. A study in adult men in the general population reported an inverse link between leptin and bone mineral density, in favour of a role of this hormone in bone mass regulation [40]. In dialysis patients, a negative relationship was found between serum leptin and PTH levels, in keeping with this role of leptin [41].

Considering this body of evidence, the possibility to modulate serum leptin levels is attractive in the search for strategies to reduce cardiac risk and improve bone quality. Of interest, we found that treatment with sevelamer-HCl, but not with calcium acetate, led to a significant decrease in serum leptin, confirming the results reported by Vlassara et al. [42] in diabetic patients with CKD. The mechanisms by which sevelamer-HCl is able to decrease leptin are still unknown. Nevertheless, our findings of a simultaneous decrease in serum leptin and FGF-23 are in agreement also with the study of Tsuji et al. [43] who showed that leptin stimulated FGF-23 expression by rat osteoblasts maintained in culture, and that intraperitoneal administration of leptin in mice increased the serum FGF-23 levels and bone FGF-23 mRNA expression. This suggests a FGF-23 regulation by leptin, compatible with the hypothesis that the reduction of serum FGF-23 observed in sevelamer-HCl treated patients was an indirect effect via leptin reduction through sevelamer.

However, the design of our study does not allow us to drive definitive conclusions regarding the mechanisms by which sevelamer-HCl modulates serum leptin, FGF-23 and sclerostin. One could speculate that sevelamer-HCl exerts its main effect through the well-known attenuation of oxidative stress and/or inflammation [44, 45] Another possibility is a decrease in the level of uremic toxins. In a preclinical study comparing the
effects of lanthanum carbonate and sevelamer-HCl on vascular calcification and bone remodelling, the presence of nitrotyrosine in atheromatous lesions, a marker for oxidative stress, was reduced by sevelamer-HCl treatment. In the same study, proteomic analysis of mouse sera treated by sevelamer-HCl revealed that several peptides were modified by CKD. The height of one of the identified peptide peaks was significantly reduced towards normal by sevelamer-HCl, but not by lanthanum carbonate administration. In addition, sevelamer-HCl treatment led to a normalization of bone formation and mineralization [44]. In another study in haemodialysis patients, treatment with sevelamer over 8 weeks was associated with increase in serum fetuin-A levels, without decrease in systemic inflammation [46]. Of note, in our study we did not find a change in serum C-reactive protein levels, but the sample size may have been too small to identify significant changes.

This study has several limitations. First of all, this was a post hoc analysis of a 6-week clinical trial with a relatively small number of patients. Second, changes in serum levels of bone-derived remodelling markers were not confirmed by bone histomorphometric analysis. Third, we did not perform an analysis of the molecular mechanisms of sevelamer-HCl action. Finally, based on current available information, it is premature to claim that reducing serum levels of leptin and sclerostin results in beneficial effects in CKD, as well as regarding the observed increase in the eGFR. Nevertheless, this is the first study involving CKD patients at early stages that describes a reduction in both serum leptin and sclerostin levels under phosphate binder treatment, in particular sevelamer-HCl.

In conclusion, our findings suggest that early CKD stages go along with an impairment of Wnt pathway and dysregulation of energy-regulating hormones. These disturbances, together with impaired bone remodelling, could be ameliorated by phosphate binder treatment, especially by sevelamer-HCl. This clinical study provides new insight into the pathophysiological understanding of early changes in CKD-MBD. However, the potentially beneficial findings need to be confirmed by long-term studies of bone quality and cardiovascular outcomes in this patient population.

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CONFLICT OF INTEREST STATEMENT

Genzyme Corporation provided the funding for this trial. The investigators were solely responsible for the design, conduct, analysis and publication of the trial. There were no restrictions on publications, and all data were maintained and analyzed solely by the authors. Drs. Canziani, Carvalho, Jorgetti and Moysés report have received consulting fees and research grants from Genzyme.

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