Effects of Thyroparathyroidectomy, Exogenous Calcium, and Short-Term Calcitriol Therapy on the Growth Plate in Renal Failure

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Abstract. Several factors have been implicated in the development of adynamic bone, including the use of calcium-containing phosphate binding agents, aggressive calcitriol therapy, and parathyroidectomy. To evaluate the effects of these interventions on the growth plate, weanling rats underwent sham nephrectomy (Control, n = 10) and 5/6 nephrectomy (Nx). In the nephrectomized group, animals underwent (a) thyroparathyroidectomy (Nx-TPTX, n = 7), (b) received exogenous calcium (Nx-Calcium, n = 10), (c) received short-term calcitriol therapy (Nx-D, n = 10), or (d) nephrectomized control (Nx-Control, n = 10). Higher serum calcium and lower PTH levels were demonstrated in Nx-Calcium and Nx-D animals. A decline in growth was demonstrated in Nx-Calcium and Nx-TPTX accompanied by shorter tibial lengths. The width of the growth plate was wider in Nx-Calcium animals due to an increase in the width of the hypertrophic zone and a decrease in the proliferative zone; these changes were accompanied by an impairment of chondroclastic resorption, lower gelatinase B/MMP-9 activity, decline in insulin-like growth factor-I (IGF-I) receptor, and lower histone-4 mRNA expression. Such findings in the growth plate, may partially contribute to the diminution of growth in these animals. Although growth was impaired in the Nx-TPTX animals, there were no significant changes demonstrated in the growth plate cartilage. Histone-4 transcripts, IGF-I receptor expression, and histochemical staining for chondroclasts were decreased in Nx-D animals. Thus, treatments used in the management of secondary hyperparathyroidism in renal failure have diverse effects on the growth plate of the young skeleton, and concurrent use of these interventions needs further evaluation.

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The prevalence of adynamic bone disease has increased in both adult and pediatric patients with chronic renal failure. The use of large doses of exogenous calcium as phosphate binding agents, aggressive calcitriol therapy, presence of diabetes, and parathyroidectomy may contribute to the development of adynamic bone in these patients (1,2).

Adynamic bone is characterized by low to normal serum intact parathyroid hormone levels (PTH), low alkaline phosphatase, and episodes of hypercalcemia (3,4). In the skeleton, there is diminished bone formation rate, a decrease in osteoblast and osteoclast number, and a decline in osteoid formation (4–6). Linear growth has been reported to decline in prepubertal children who developed adynamic bone during treatment of secondary hyperparathyroidism with high intermittent doses of calcitriol (7). Reductions in tibial length have been demonstrated in nephrectomized rats given exogenous calcium accompanied by reductions in growth (8). Iwasaki et al. (9) have reported that parameters of bone formation were decreased in nephrectomized rats that underwent thyroparathyroidectomy and were given high-calcium diet. In addition, diminution of body weight and inhibition of skeletal growth were reported in young rats with normal renal function after parathyroidectomy (10).

Adynamic bone may evolve during the course of a variety of treatment modalities for secondary hyperparathyroidism; however, there is little information available on the precise mechanism on how parathyroidectomy affects chondrocyte proliferation and chondrocyte hypertrophy in renal failure. The current study was undertaken to evaluate and compare the effects of parathyroidectomy, exogenous calcium, and short-term calcitriol therapy on the growth plate of young animals with renal failure.

Materials and Methods
Forty-seven male weanling Sprague-Dawley rats, weighing 60 to 70 g (Harlan Laboratories, Madison, WI) were obtained and housed in individual cages at constant temperature with a 12-h light and 12-h dark cycle. All procedures were reviewed and approved by the Research Animal Resource Center at the University of Wisconsin, Madison, WI. The animals had free access to drinking water and standard rodent diet containing 23.4% protein, 0.6% calcium, and 0.6% phosphorus (Purina Mills, Indianapolis, IN). After 24 h of acclimatization, 37 rats underwent the first stage of a two-stage 5/6 nephrectomy (Nx) under ketamine and xylazine anesthesia (11). The second stage nephrectomy was completed 7 d later, and seven animals underwent thyroparathyroidectomy (TPTX) at the same time (12). Ten rats underwent sham nephrectomy in two stages corresponding to the time of the two-stage 5/6 nephrectomy.
All animals were weighed and body length was determined in sedated animals by measuring the distance from the tip of the nose to the end of the tail. The 6-wk study period began 24 h after completion of the second surgery. Ten sham-nephrectomized (Intact) animals, and 27 nephrectomized animals (Nx) continued to ingest standard rodent diet. Intact animals (Intact-Control, n = 10) were pair-fed with animals that have undergone subtotal nephrectomy by providing the amount of food each day to intact rats that had been consumed the previous day by nephrectomized animals. Ten nephrectomized animals fed regular rodent diet served as Nx-Control group. Another group of nephrectomized animals were fed high-calcium diet (Nx-Calcium) consisting of 23.4% protein, 2.0% calcium, and 0.68% phosphorus. Blood was obtained for serum calcium, phosphorus, creatinine, and parathyroid hormone (PTH). The proximal tibiae were excised, and tibial length was measured at equally spaced intervals using an image analysis software (Kontron Instruments Ltd Elektronik 200, Hallbergmoos, Germany) (11). Data are expressed as the number of silver grains overlying each chondrocyte profile was counted and expressed as percent of the number of positive cells to the total number of cells in the appropriate growth plate zone where the mRNA expression is localized.

Serum Biochemical Determinations

Serum was obtained by centrifugation, and samples were stored at -70°C until biochemical assays are done. Serum urea nitrogen, creatinine, calcium, and phosphorus levels were measured using standard laboratory methods (11). Serum intact PTH levels were measured using the Rat Intact PTH ELISA assay kit (Immutopics Inc, San Clemente, CA).

Growth Plate Morphometry

For morphometric analysis, three 5-μm sections of bone were obtained from each tibia, stained with hematoxylin and eosin, and counterstained with azure A. Sections were viewed by light microscopy at ×40 using a Leitz Leica microscope (W. Nushbaum Inc, McHenry, IL), and images were captured onto a computer monitor using a Hitachi HV-C20 CCD video camera control unit (Hitachi Denshi Ltd, Tokyo, Japan). The total width of the growth plate at the proximal end of each tibia was measured at equally spaced intervals using an image analysis software (Kontron Instruments Ltd Elektronik 200, Hallbergmoos, Germany) (13). The widths of the zones occupied by hypertrophic chondrocytes and proliferative chondrocytes were measured by the same method.

Quantification of In Situ Hybridization Signals

Slides were viewed at ×100 by bright field microscopy using a Leitz Leica microscope (W. Nushbaum Inc, McHenry, IL). The number of silver grains overlying each chondrocyte profile was counted using an image analysis system (Kontron Instruments Ltd Elektronik 200, Hallbergmoos, Germany) (11). Data are expressed as the number of silver grains/1000 μm² of cell profile.

For the non-radioactive in situ hybridization, the number of cells expressing the specific mRNA was counted and expressed as percentage of the number of positive cells to the total number of cells in the appropriate growth plate zone where the mRNA expression is localized. The mRNA expression for MMP-9/gelatinase B was quantified by measuring the area with positive staining, and results are expressed as labeled area over the total tissue area in the chondro-osseous junction.

Immunohistochemistry (Bcl-2, Bax, Proliferating Cell Nuclear Antigen [PCNA], TUNEL, TRAP)

Immunohistochemistry was performed using methods described previously (13) with the following primary antibodies: Bcl-2 (monoclonal) 1:4 in blocking buffer or Bax (monoclonal) 1:100 in blocking buffer (Santa Cruz Biotechnology, San Diego, CA) and incubated at 4°C overnight in a humidified chamber. For PCNA staining, the tissues were immunostained with the mouse anti-PCNA antibody (Zymed Laboratories, South San Francisco, CA) using the protocol from the company. For quantification, the number of cells expressing Bcl-2 protein, Bax protein, or PCNA was counted and expressed as percentage of the total number of cells (nucleated cells for PCNA) in the appropriate zone in the growth plate.
For the TUNEL assay, the specimens were labeled using the kit from Intergen (Gaithersburg, MD). The number of TUNEL-positive cells were counted and expressed as percentage of the total number of terminal chondrocytes in each section.

Histochemical staining for tartrate-resistant acid phosphatase (TRAP) was done using methods reported previously (11). Image analysis was done in tissue sections viewed at ×40, projected onto the computer screen, and the number of TRAP-positive cells in the chondro-osseous junction was quantified and expressed as number of cells per area of the growth plate in the chondro-osseous junction.

Statistical Analyses
All results are expressed as mean values ± 1 SD. Data were evaluated by one-way ANOVA, and comparisons among groups were done using Bonferroni/DUNN post-hoc tests using the StatView statistical software (SAS Institute, Cary, NC). The Pearson product moment correlation coefficient was performed to evaluate the relationship between two numerical variables. For all statistical tests, probability values less than 5% were considered to be significant.

Results
Serum creatinine and serum urea nitrogen levels were elevated in the nephrectomized (Nx) animals compared with animals with normal renal function (Table 1). Serum calcium levels were higher, and intact PTH levels were lower in the Nx-Calcium and Nx-D groups (Table 1). Serum phosphorus levels were 20% lower in the Nx-Calcium animals compared with the Nx-Control group (Table 1). PTH levels were higher by 64% in the Nx-Control group compared with Intact-Control and higher by 84% compared with Nx-TPTX animals (Table 1).

In the Nx-TPTX animals, the serum calcium and serum phosphorus levels obtained 2 d after the thyroparathyroidectomy procedure were much lower than baseline, 10.9 ± 0.4 mg/dl to 8.7 ± 0.9 mg/dl, and 11.7 ± 0.9 mg/dl to 8.9 ± 0.9 mg/dl (P < 0.01), respectively. In contrast, both serum creatinine and urea nitrogen levels already increased after 2 d of nephrectomy in these animals, 0.3 ± 0.05 mg/dl and 25 ± 3.1 mg/dl at baseline to 0.7 ± 0.08 mg/dl and 67 ± 11 mg/dl (P < 0.01).

Although baseline weights were similar in all groups, there was a 15% decline in weight gain in the Nx-TPTX, Nx-Calcium, and the Nx-D animals (Table 2). Conversely, the Nx-Control and the Intact-Control groups had comparable increases in weight at the end of the study. Mean body length measurements at the end of the study period was 20% shorter in Nx-TPTX animals and 9% shorter in the Nx-Calcium group compared with Nx-Control, Nx-D, and Intact-Control (Table 2). Tibial length measurements were shorter in Nx-TPTX and Nx-Calcium animals, 3.8 ± 0.1 cm and 3.9 ± 0.09 cm, respectively, when compared with Nx-Control and Intact-Control groups, 4.0 ± 0.1 cm (P < 0.02). In the Nx-D animals, the average tibial length was 4.0 ± 0.1 cm. There was no significant correlation between the body length measurements and the serum IGF-I levels at the end of the study period, R = −0.15.

The width of the growth plate cartilage was widest in the calcium loaded animals (Nx-Calcium), and this expansion was primarily due to the increase in the zone occupied by the hypertrophic chondrocytes and a small reduction in the area of the proliferating chondrocytes (Figure 1). In the Nx-TPTX animals, the growth plate was wider compared with the Nx-Control, Nx-D, and Intact-Control groups, but the values did not reach statistical significance. The widths of the hypertrophic and proliferative zones were comparable in the Nx-TPTX, Nx-D, Nx-Control, and Intact-Control animals (Figure 1).

Several markers of chondrocyte proliferative activity were evaluated in the growth plate cartilage. Type II collagen expression localized to both proliferative and hypertrophic zones did not differ in all groups. Immunohistochemical staining for PCNA expression localized to the proliferative zone was similar in all nephrectomized animals, 17 ± 10% in Nx-Control, 15 ± 7% in Nx-Calcium, and 13 ± 9% in Nx-TPTX, and 10 ± 9% in Nx-D animals (P = NS), but the staining was 50% less compared with the Intact-Control group, 35 ± 20% (P < 0.004).

Histone-4 mRNA expression, which is expressed during the S-phase of the cell cycle (15), was confined to the lower proliferative and upper hypertrophic zones and declined in the Nx-Calcium and Nx-D groups, 47 ± 10%, compared with the

Table 1. Serum biochemical parameters in all groups

<table>
<thead>
<tr>
<th></th>
<th>Intact-Control n = 10</th>
<th>Nx-Control n = 10</th>
<th>Nx-Calcium n = 10</th>
<th>Nx-TPTX n = 7</th>
<th>Nx-D n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>9.7 ± 0.4</td>
<td>9.7 ± 0.6</td>
<td>11 ± 0.6a</td>
<td>9.0 ± 1.1</td>
<td>11 ± 1.0a</td>
</tr>
<tr>
<td>Serum phosphorus (mg/dl)</td>
<td>9.6 ± 1.0</td>
<td>9.1 ± 1.0</td>
<td>7.3 ± 0.9b</td>
<td>9.3 ± 1.1</td>
<td>9.0 ± 1.0</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.3 ± 0.3</td>
<td>0.9 ± 0.3c</td>
<td>0.8 ± 0.3c</td>
<td>0.7 ± 0.5c</td>
<td>0.7 ± 0.4c</td>
</tr>
<tr>
<td>Serum urea nitrogen (m/dl)</td>
<td>16 ± 1.5</td>
<td>61 ± 3.6c</td>
<td>53 ± 3.7c</td>
<td>51 ± 3.7c</td>
<td>52 ± 4.3c</td>
</tr>
<tr>
<td>Serum iPTH (pg/ml)</td>
<td>97 ± 7.8</td>
<td>273 ± 10d</td>
<td>8.7 ± 2.6a</td>
<td>41 ± 5.0</td>
<td>7.8 ± 3.0a</td>
</tr>
</tbody>
</table>

a P < 0.001 versus Nx-Control, Nx-TPTX, Intact-Control.
b P < 0.001 versus Nx-Control, Nx-TPTX, Nx-D, Intact-Control.
c P < 0.001 versus Intact-Control.
d P < 0.001 versus Nx-Calcium, Nx-TPTX, Nx-D, Intact-Control.

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Table 2. Anthromorphic measurements in all groups

<table>
<thead>
<tr>
<th></th>
<th>Intact-Control</th>
<th>Nx-Control</th>
<th>Nx-Calcium</th>
<th>Nx-TPTX</th>
<th>Nx-D</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 7)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td>Gain in weight(a) (g)</td>
<td>193 ± 4.6</td>
<td>183 ± 4.0</td>
<td>157 ± 4.5(b)</td>
<td>149 ± 4.2(b)</td>
<td>166 ± 5.6(b)</td>
</tr>
<tr>
<td>Gain in length(b) (cm)</td>
<td>12 ± 1.0</td>
<td>12 ± 0.9</td>
<td>11 ± 1.1(c)</td>
<td>9.7 ± 1.0(d)</td>
<td>12 ± 1.0</td>
</tr>
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</table>

\(a\) Difference in weight or length obtained at the beginning and end of the study period (6 wk).
\(b\) \(P < 0.02\) versusNx-Control, Intact-Control.
\(c\) \(P < 0.02\) versusNx-Control, Nx-D, Intact-Control.
\(d\) \(P < 0.02\) versusNx-Control, Nx-Calcium, Nx-D, Intact-Control.

Figure 1. The total width of the epiphyseal growth plate cartilage in the proximal tibia (□), proliferative zone (□), and hypertrophic zone (■) in Intact-Control, Nx-Control, Nx-Calcium, Nx-TPTX, and Nx-D animals. \(^{\ast} P < 0.001\) versus Nx-Control, Nx-TPTX, Nx-D, and Intact-Control. The corresponding photomicrographs are shown above. Magnification, \(\times 20\).
A significant decline in IGF-I receptor mRNA transcripts was demonstrated in the Nx-Calcium and Nx-D animals, 50 ± 8% compared with Nx-TPTX, Nx-Control, and Intact-Control groups, 70 ± 12% (P < 0.03; Figure 3). The PTH/PTHrP receptor and type X collagen mRNA expression in the growth plate cartilage did not differ in all groups.

There was a 60% decline in the gelatinase B/MMP-9 mRNA expression in the chondro-osseous junction of Nx-Calcium animals compared with the Nx-TPTX and Intact-Control animals, but there was a threefold increase in gelatinase B/MMP-9 expression in the Nx-Control animals (Figure 4). The gelatinase B mRNA expression correlated well with the serum PTH levels in all animals at the end of the study period: R = 0.83, P < 0.005.

TUNEL staining for apoptotic cells remained similar in all groups, 7 ± 4.5% in Nx-Calcium, Nx-D, and Intact-Control groups, and 10 ± 4% in Nx-Control and Nx-TPTX (P = NS). Likewise, the expression for the angiogenic factor, vascular endothelial growth factor (VEGF) did not differ in the nephrectomized and Intact-Control animals. The immunoreactivity of apoptosis inhibitor, Bcl-2, and apoptosis inducer, Bax, and the ratio between Bcl-2 and Bax were equivalent in all groups.

Histochemical staining for tartrate-resistant acid phosphatase (TRAP) in chondroclasts located in the chondro-osseous junction was much less in the Nx-Calcium and Nx-D groups, 11 ± 4.8 cells/area and 9 ± 3.7 cells/area, compared with Nx-Control, Nx-TPTX, and Intact-Control animals, 18 ± 5, 21 ± 6, and 28 ± 4 cells/area, respectively (P < 0.001; Figure 5). Only TRAP staining in the chondro-osseous junction was evaluated and quantified, and TRAP staining found in the primary spongiosa was not included.

Discussion

In the current study, therapeutic interventions to control secondary hyperparathyroidism such as parathyroidectomy and the administration of exogenous calcium are associated with a decrease in bone elongation and a diminution in body growth. Although the effects on skeletal growth are almost similar, the histologic findings on the growth plate were different in calcium-treated animals and in animals that underwent thyroparathyroidectomy. The body length and tibial length measurements were shortest in the Nx-TPTX group at the end of the study period without any significant alterations in the growth plate cartilage. These findings in the Nx-TPTX animals may in part be secondary to a lower caloric intake after the thyroparathyroidectomy procedure. The average daily caloric intake in all animals was comparable, but the animals that underwent thyroparathyroidectomy always consumed the lowest amount. Keil et al. (10) have demonstrated a considerable decline in body weight and femur length in parathyroidectomized animals with normal renal function, and these changes were thought to be secondary to the alterations in mineral metabolism and lower food consumption, which may have directly or indirectly resulted from lower circulating PTH. The thyroparathyroidectomized rats in the study had comparable serum calcium and serum phosphorus levels but much lower parathyroid hormone levels compared with nephrectomized control animals at the end of the study period. The combination of the smaller food consumption and the lower serum PTH levels may have contributed to the decline in bone growth in the Nx-TPTX group. The growth plate of the thyroparathyroidectomized animals in the current study showed a mildly lower gelatinase B/MMP-9 expression in the chondro-osseous junction compared with the Intact-Control group; such change, however, was not statistically significant and may not fully explain the significant impairment in skeletal growth. The effects of thyroparathyroidectomy on the expression of other regulators of chondrocyte differentiation (i.e. Indian hedgehog, core binding factor1, etc) were not evaluated in the current study.

It was expected that the serum PTH levels will be low in the thyroparathyroidectomized group; however, animals given exogenous calcium and those treated with calcitriol had the lowest serum PTH levels. Recent studies of Gcm2-deficient mice that lack parathyroid glands exhibited mildly abnormal biologic hypoparathyroidism, but the serum PTH levels were identical to the wild-type mice and parathyroidectomized wild-type animals (16). Some of the auxiliary mechanisms for the regulation of serum calcium may be located in the thymus gland and the hypothalamus in absence of the parathyroid glands (16). The Gcm2-deficient mice had an increase in trabecular bone thickness and a decrease in osteoclast and osteoblast number (16). In our current study, the intensity of TRAP staining was similar in the Nx-TPTX and the Intact-Control animals and lowest in the calcium-loaded and calcitriol-treated rats. In addition, the chondroclast number in the chondro-osseous junction was higher in the Nx-TPTX animals compared with the Nx-Calcium and Nx-D groups. These findings may, in part be secondary to the higher serum PTH levels demonstrated in the Nx-TPTX animals. Exogenous calcium and calcitriol given to nephrectomized animals may exert a generalized inhibitory effect on the parathyroid glands and any other accessory glands which may participate in PTH synthesis and secretion.

Serum 1,25(OH)2D3 levels were not obtained in this study, but previous studies have reported normal levels in parathyroidectomized animals (9). There were no evident inhibitory effects on chondrocytic proliferative activity, chondrocyte apoptosis, and the expression of the angiogenic factor, VEGF, in the thyroparathyroidectomized animals. Russell et al. (17) have demonstrated that parathyroidectomy did not did not alter thymidine incorporation onto DNA in proliferating cells.

Some the changes described in the growth plate of animals that received exogenous calcium, such as widening of the growth plate, increase in the area occupied by the hypertrophic chondrocytes, decrease in gelatinase B, and lower TRAP staining, have been reported previously in our earlier experiments (8). The same changes in the growth plate are demonstrated in the current experiments despite the lower dietary calcium content compared with our previous study. Iwasaki et al. (9) have demonstrated that rats fed 2.0% calcium and 1.0% phosphorus (similar dietary calcium content in the current study) had bone histomorphometric values comparable with those described in
Adynamic bone. Although the effects of calcium loading on chondrocyte differentiation and ossification in the current study were similar to our earlier reports (8), adverse effects of exogenous calcium on the proliferative activity of the growth plate chondrocytes have not been demonstrated in our previous study. There was a significant decrease in the area occupied by the proliferating chondrocytes accompanied by a significant decline in histone-4 and IGF-I mRNA expression; these changes were also demonstrated in the calcitriol-treated animals despite the short duration of treatment. Exogenous calcium may directly or indirectly affect the S phase of chondrocyte proliferation because the PCNA staining did not decline in these animals. The decrease in the proliferative zone of the growth plate after calcium loading was not demonstrated in our previous experiment (8), and this may be secondary to the younger age of the animals in the current study at the end of the study period. Exogenous calcium administration in nephrectomized animals may have more adverse effects on cell proliferation in the growth plate of younger animals.

Insulin-like growth factor I and its receptor play important roles in chondrocyte proliferation and chondrocyte hypertrophy in the growth plate cartilage. Previous experiments have shown that renal failure, per se led to a reduction in IGF-I expression in the growth plate and in the liver with a concomitant elevation in IGF binding protein concentration (18,19). In our current study, however, the IGF-I receptor expression in

Figure 2. Histone-4 mRNA expression (denoted by arrows) localized in the lower proliferative zone and in the upper hypertrophic zone in Intact-Control, Nx-Control, Nx-Calcium, Nx-TPTX, and Nx-D animals; magnification, ×20. The number of positive cells was quantified and expressed as percentage of positive cells to total number of cells, *P < 0.02.
the growth plate did not differ between the nephrectomized and intact control animals, and this may be due to the differences in the age of the rats used. IGF-II expression, which is predominantly found in the growth plate of younger rats, and the expression of IGF binding proteins were not evaluated in the current study.

In the calcium-loaded nephrectomized animals, however, there was a significantly lower expression of IGF-I receptor. Such findings are in contrast to earlier in vitro experiments that demonstrated that high extracellular calcium concentration stimulated DNA synthesis in MC3T3-E1 osteoblastic cells, osteosarcoma cells, human vertebrae, and human articular cartilage cells mediated in part by a considerable increase in IGF-I and IGF-II secretion, and IGF-I receptor expression (20,21). In the presence of renal failure, exogenous calcium loading may directly or indirectly inhibit IGF-I receptor expression or it may alter the synthesis and expression of the IGF-binding proteins. IGF binding proteins regulate the actions of IGF-I by prolonging its half-life, directing its distribution or modifying the interaction of IGF-I with its receptor (22). Yoo and co-workers have described that insulin-like growth factor binding proteins demonstrated inhibitory effects on the osteogenic actions of IGF-I during calcium administration to calcium-depleted rats with normal renal function (23). Further studies are needed to evaluate whether exogenous calcium affects the expression of inhibitory IGF binding proteins in the growth plate of nephrectomized animals.

Although, serum IGF-I levels were not performed in this

Figure 3. Insulin-like growth factor-I (IGF-I) receptor mRNA in the lower proliferative and the hypertrophic zone as denoted by arrows in Intact-Control, Nx-Control, Nx-Calcium, Nx-TPTX, and Nx-D animals; magnification, ×50. The number of positive cells was quantified and expressed as percentage of positive cells to total number of cells, *p < 0.03.
study, our unpublished experiments have demonstrated equivalent serum IGF-I levels in calcium-loaded nephrectomized animals and in the Nx-Control group. Thus, serum IGF-I concentration does not influence IGF-I receptor expression in chondrocytes. Tonshoff et al. (24) have also described that a decrease in hepatic IGF-I expression in the liver did not correlate with a decline in the plasma IGF-I levels in rats with renal failure. Thus, serum levels of biochemical markers that may affect bone growth such as IGF-1 or intact PTH do not correlate well with the actual measurements of bone growth in nephrectomized animals.

The widening of the hypertrophic zone in the calcium-loaded animals may be secondary to a decrease in gelatinase B/MMP-9 and chondroclast activity in the chondro-osseous junction of the calcium-loaded animals. It was surprising to note, however, that in the current study, gelatinase B/MMP-9 expression increased in the nephrectomized control group compared with the Intact-Control animals. These changes may be
explained by the higher serum PTH levels in the nephrectomized control animals and the younger age of the rats at the end of the study period. A significant correlation between the serum intact PTH levels and the expression of gelatinase B/MMP-9 in the chondro-osseous junction of the growth plate is demonstrated in the current study. Previous studies have demonstrated that PTH administration stimulates the expression of MMP-9 in the rat long bone and in the mononuclear and osteoclastic cells in the fetal rat limb (25,26). The lower serum PTH levels in the calcium-loaded animals may have played a role in the lower gelatinase-B/MMP-9 activity in the chondro-osseous junction and may have contributed to the delay in ossification. However, in the calcitriol-treated animals, there were no changes demonstrated in gelatinase B/MMP-9 expression despite lower serum PTH levels and a decline in chondroclastic activity. Thus, exogenous calcium may have directly inhibit gelatinase B/MMP-9 activity, and these changes are probably not mediated by PTH.

Earlier experiments have demonstrated that calcitriol has a dose-dependent anti-proliferative effect on chondrocyte prolif-

![Figure 5. Histochemical staining for tartrate-resistant acid phosphatase (TRAP) in the chondro-osseous junction (denoted by arrows and red stain) in Intact-Control, Nx-Control, Nx-Calcium, Nx-D, and Nx-TPTX animals; magnification, ×50. The number of TRAP-positive cells in the chondro-osseous junction was counted and expressed as number of cells per area, *P < 0.001 versus Nx-Control, Nx-TPTX, and Intact-Control.]
eration in young animals with normal renal function (27). A
decrease in histone-4 and IGF-I receptor gene expression were
demonstrated in our study only after 10 d of calcitriol therapy
in nephrectomized animals comparable to the findings
demonstrated in the calcium-loaded animals. Nonetheless, there were
no significant changes on bone growth, PCNA or type II collagen expression in the Nx-D animals probably secondary to the
short duration of treatment. Calcitriol, like exogenous calcium,
may directly inhibit the initiation of the cell cycle af-
flecting only the histone gene expression. Scharla et al. (28)
have also demonstrated that calcitriol increased the expression of
IGF binding protein-4, which has inhibitory effects on mouse osteoblast function. In addition, other experiments have
shown that calcitriol blunted the growth response to growth
hormone therapy in uremic animals by decreasing the IGF-I
expression in the growth plate cartilage (29). Longer treatment
with calcitriol in nephrectomized animals may result in shorter
bone growth because of its inhibitory effects on cell proliferation
and IGF-I expression.

Other factors that may contribute to alterations in endochon-
dral bone formation in nephrectomized animals, such as met-
abolism-related and significant loss of urinary protein, were not
evaluated. Our unpublished results in previous experiments
have shown that the mean blood pH of nephrectomized rats and
animals with normal renal function was comparable at 7.30 ±
0.02.

Exogenous calcium loading, parathyroidectomy and short-
term calcitriol therapy performed in our study affected skeletal
growth and the growth plate by different mechanisms. These
findings are especially important in children because an earlier
study has described linear growth impairment in prepubertal
children who received 12 mo of high-dose intermittent calcit-
riol therapy and exogenous calcium as phosphate binding
agents (7). Our current results demonstrate that calcium loading
seems to exert more inhibitory effects on chondrocyte proliferation and ossification than short-term calcitriol therapy
alone in nephrectomized young rats. The common practice of
combining high-dose calcium therapy and aggressive calcitriol
treatment in the management of children with secondary hy-
perparathyroidism should be reevaluated in the young and
growing animals. Parathyroidectomy affected bone elongation
and body growth but there were no significant changes evident
in the growth plate compared to the nephrectomized control
group. Further studies are needed to address issues regarding
appropriate doses and proper administration if these therapeu-
tic interventions will continue to be used concurrently in children with renal failure and secondary hyperparathyroidism.

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References
1. Yamamoto T, Ozono K, Miyauchi A, Kasayama S, Kojima Y,
Shima M, Okada S: Role of advanced glycation end products in
adynamic bone disease in patients with diabetic nephropathy.
2. Yajima A, Ogawa Y, Ikehara A, Tominao T, Inou T, Otsubo O:
Development of low-turnover bone disease after parathyroidect-
Vlachojannis J, Malluche HH: Evidence for abnormal calcium
homeostasis in patients with adynamic bone disease. Kidney Int
46: 855–861, 1994
EB, Gokal R: Histological, radiological and biochemical features
of the adynamic bone lesion in continuous ambulatory peritoneal
5. Goodman WG, Ramirez JA, Belin TR, Choi Y, Gales B, Segre
GV, Salusky IB: Development of adynamic bone in patients with
secondary hyperparathyroidism after intermittent calcitriol ther-
6. Malluche HH, Monier-Faugere M-C: Risk of adynamic bone
7. Kuizon BD, Goodman WG, Juppner H, Boechat I, Nelson P,
Gales B, Salusky IB: Diminished linear growth during intermit-
tent calcitriol therapy in children undergoing ccpd. Kidney Int 53:
205–211, 1998
8. Sanchez CP, Kuizon BD, Abdella PA, Juppner H, Salusky IB,
Goodman WG: Impaired growth, delayed ossification, and re-
duced osteoclastic activity in the growth plate of calcium-sup-
plemented rats with renal failure. Endocrinology 141: 1536–
1553, 2000
Kurokawa K, Fukagawa M: Substantial and independent contri-
bution of renal dysfunction on the pathogenesis of adynamic
2001
bone growth and composition in the young rat. Growth 38:
519–527, 1974
11. Sanchez CP, Salusky IB, Kuizon BD, Abdella P, Juppner H,
Gales B, Goodman WG: Growth of long bones in renal failure:
Roles of hyperparathyroidism, growth hormone and calcitriol.
12. Goodman WG: Thyroparathyroidectomy modifies the skeletal
response to aluminum loading in the rat. Kidney Int 31: 923–929,
1987
13. Sanchez CP, He Y-Z: Alterations in the growth plate cartilage
in rats with renal failure receiving corticosteroid therapy. Bone
30: 692–698, 2002
14. Lee K, Deeds JD, Bond AT, Juppner H, Abou-Samra AB, Segre
GV: In situ localization of PTH/PTHrP receptor mRNA in the
of cell cycle progression: The histone gene is a paradigm for the
G1/S phase and proliferation/differentiation transitions. Cell Bi-
ology Int 20: 41–49, 1996
16. Gunther T, Chen Z-F, Kim J, Priemel M, Rueger JM, Amling M,
Moseley J, Martin TJ, Anderson DJ, Karnsley G: Genetic
ablation of parathyroid gland reveals another source of parathy-


29. Facincani I, Salusky IB, Sanchez CP, Cambay EL, Goodman WG, Kuizon BD: Calcitriol (D) inhibits growth hormone receptor (GHR) and IGF-I expression induced by growth hormone (GH) in growth plate (GP) of rats with osteitis fibrosa (OF) [Abstract]. J Am Soc Nephrol 12: 742A, 2001