

# Growth hormone treatment prevents osteoporosis in uremic rats

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**Summary.** Introduction: Growth hormone (GH) is responsible for longitudinal bone growth. GH-receptor in the growth plate was found to be decreased in chronic renal insufficiency. A therapeutic use of GH in chronic renal insufficiency is not established. The current study aims to clarify the effects of GH treatment on bone metabolism in a uremic rat model. Methods: Sprague Dawley rats were subjected to subtotal surgical renal ablation (SNX) or sham operation. SNX rats were randomized into 4 groups: treated with different doses of GH (1.5, 4.0, or 10.0 mg/kg) or vehicle after 10 weeks of uremia and treated for 6 weeks. Bone and renal morphology was evaluated: bone density, thickness of spongiosa, osteoblast surface, osteoid volume, osteoclast quantity, and resorptive volume. Results: GH treatment resulted in a decrease of resorption area and lower number of osteoclasts. Osteoid volume, number of osteoblasts, percentage of active osteoblasts, thickness of the growth plate and mean cortical width increased. GH receptor (GHR) protein expression increased in GH treated rats. IGF-1 expression was decreased in osteoblasts and chondroblasts of SNX-V rats and increased following GH treatment. The TGF- $\beta$  expression was down regulated in SNX+V group in osteocytes and chondroblasts as compared to sham operated animals. The down regulation was prevented in treated animals irrespective of the dose given. Conclusions: Treatment with GH in uremic animals increased bone density to the levels of non-uremic controls. Thus GH seems to have a potential of preventing renal osteodystrophy.

**Key words:** Osteoporosis, Uremia, Growth hormone

## Introduction

Bone remodelling is a dynamic process comprising a balance between bone synthesis and bone resorption, which can be strongly affected by a decline of the glomerular filtration rate (GFR) (Fournier et al., 1994). Renal bone disease represents a wide spectrum of skeletal abnormalities ranging from high-turnover bone lesions seen in patients with uncontrolled hyperparathyroidism to the profound reduction of bone metabolism seen in patients with adynamic bone disease. The development of skeletal abnormalities does not appear to depend on the specific type of renal disease. The underlying pathogenetic mechanisms of renal bone disease are multifactorial. Alteration of vitamin D metabolism, disturbances of calcium and phosphate balance, failure of metabolism and action of parathyroid hormone with limited renal clearance and increased parathormone secretion lead to alteration of bone metabolism in patients with chronic renal disease.

Osteoporosis is common in patients with renal failure. The use of standard therapies for osteoporosis in patients with CKD is highly controversial. The impact of agents considered, including the bisphosphonates, estrogens, selective estrogen receptor modulators (SERMs), calcitonin, and androgens/anabolic steroids, is uncertain, as is the effect of these treatments on bone turnover, bone quality, and fracture risk. None of these agents has licensed indications within the field of renal osteodystrophy in the United States or in most European countries.

Growth hormone (GH) is responsible for longitudinal bone growth through its direct action on

epiphyseal chondrocytes and through the induction of IGF-1 (Isaksson et al., 1982). GH also plays an important role in regulating bone remodelling. GH substitution increases and thus normalizes bone mineral density in patients with hypopituitarism, which is one of a number of arguments for GH substitution (Biermasz et al., 2004). In contrast, a possible therapeutic use of GH in idiopathic osteoporosis and glucocorticoid-induced osteoporosis is speculative and not yet established (Aloia et al., 1976).

The effect of bovine GH on the process of bone formation and bone resorption was demonstrated *in vitro* (Ueland, 2005). Bovine GH increased DNA synthesis, stimulated alkaline phosphatase activity and enhanced both type 1 procollagen mRNA expression and collagen expression in osteoblastic MC3T3-E1 cells. These effects are proven to be independent of IGF-1 activation. Resistance to GH is a well recognized complication of uremia (Tonshoff et al., 2005). However, relative GH resistance is observed in uremia while the rising GH concentration in uremia is accompanied by a decrease of serum IGF-I levels and increased IGF-BPs. This led to the idea that growth hormone and insulin-like growth factor could be manipulated to achieve benefit of growth disorders in renal failure (Roelfsema and Clark, 2001; Rabkin et al., 2005). For example, in children with CRF, despite the presence of normal or elevated GH levels, body growth is attenuated, and relatively large doses of recombinant GH are required to enhance linear growth (Zadik et al., 1998). In rats with renal failure, body growth is also impaired, and GH induced weight gain and IGF-1 expression is depressed (Schaefer et al., 2001). Impaired JAK-STAT signal transduction contributes to growth hormone resistance in chronic uremia (Schaefer et al., 2001). This resistance to GH arises because of defects at several levels in the GH-IGF-1 system (Rabkin et al., 2005; Tonshoff et al., 2005). GH receptor levels may be low, though this is not a uniform observation. GH activation of the janus kinase 2-signal transducer (JAK2) and activator of transcription (STAT) pathway is attenuated (Schaefer, et al., 2001; Rabkin et al., 2005) and this leads to reduced IGF-1 production. Furthermore there is resistance to IGF-1, the major mediator of GH action, and together with reduced IGF-1 expression it is a major cause of the attenuated linear growth.

Since GH is an important regulator of bone remodeling and is effective in normalizing bone density in hypopituitarism, we made a study of uremic rats, to determine whether treatment with recombinant GH is effective in correcting the diminished bone density that is prevalent in chronic renal failure.

## Materials and methods

### Animals

4-6 week old male, pituitary intact, Sprague-Dawley

rats weighing 140 to 170 g were entered into the study which lasted over 4 months. The animals were housed in individual cages at constant temperature with a 12-h light and 12-h dark cycle.

### Experimental design

Chronic renal failure (CRF) was induced by a two-stage 5/6 nephrectomy (SNX) procedure and sham-operated rats served as control. In the first intervention, approximately 2/3 of left kidney was removed through a flank incision. Seven days later, the right kidney was removed, subsequently resulting in a state of stable CRF. Ten weeks after the second surgical intervention, Sham or CRF rats were randomized into the following groups: 1. Sham-operated rats, vehicle only (Sham, n=7); 2. CRF rats given vehicle only (SNX+V, n=5); 3. CRF rats treated with bGH 1.5 mg/kg/day (SNX+GH 1.5, n=7); 4. CRF rats treated with bGH 4.0 mg/kg/day (SNX+GH 4.0, n=7); 5. CRF rats treated with bGH 10.0 mg/kg/day (SNX+GH 10.0, n=7).

GH therapy started after 10 weeks of uremia and lasted for 6 weeks, at which time the rats were 21 to 23 weeks old. The intervention was administered subcutaneously in three different doses (1.5, 4.0 or 10.0 mg/kg/day). All animals were weighed weekly for regular GH dose adjustment. The animals had free access to drinking water and standard rodent diet containing 23.4% protein, 0.6% calcium, and 0.6% phosphorus. Blood pressure was measured by a tail-cuff method just before GH treatment and thereafter every 3 weeks. Serum creatinine level was examined by a standard laboratory method before GH treatment and during sacrifice. After 6 weeks of GH treatment, the animals were sacrificed. Both femoral bones and kidneys were harvested after perfusion with glutaraldehyde for morphometric investigations.

### Histology

*Femoral bone* tissue was taken for histological analysis. For analysis of bone mineralization, one femur of each animal was fixed in 70% alcohol and embedded in methyl methacrylate. Longitudinal sections of the femur specimens (5  $\mu$ m thick) were cut (frontal plane), stained with Masson and Goldner, Kossa, van Gieson stainings and used for morphometry.

The second femur of each animal were fixed in buffered formalin (4%), decalcified in formic acid (4N, 15%) for 2 days and embedded in paraffin. Longitudinal sections (5  $\mu$ m thick) were cut and used for performing of immunohistochemistry.

The *kidney* was dissected in a plane perpendicular to the interpolar axis, yielding slices of 1 mm width. Ten small pieces of this kidney were selected by area-weighted sampling for embedding in Epon-Araldite. Semithin (1  $\mu$ m) and ultrathin sections (0.08  $\mu$ m) were prepared and stained with methylene blue/basic fuchsin

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or citrate/uranyl acetate, respectively. The remaining tissue slices were embedded in paraffin; 4  $\mu\text{m}$  sections were prepared and stained with hematoxylin/eosin (HE) and PAS. For immunohistological investigations, one half of the NaCl-perfused kidney was fixed in 4% buffered formaldehyde, embedded in paraffin and cut into 2  $\mu\text{m}$  thick sections. For *in-situ* hybridization, the other half of the kidney was snap frozen in liquid nitrogen-cooled isopentane.

### Morphometry

The following parameters were analyzed: trabecular bone volume (%), cortical bone volume (%), mean cortical width (mkm), trabecular thickness (mkm), osteoid volume (%), resorptive surface (%), cortical porosity (%), osteoblast and osteoclast number (per  $\text{mm}^2$  of the trabecular bone tissue), index of osteoblast activity, thickness of growth plate ( $\mu\text{m}$ ).

Index of osteoblast activity represents the percentage of trabecular bone surfaces covered by osteoid that is lined by typical cuboidal osteoblasts.

Morphometric analysis was performed using image analysis software (Image-Pro Plus, ver. 5.1.0.20, Media Cybernetics, Inc. Silver Spring, USA). Sections were viewed by light microscopy at x50, and images were captured onto a computer monitor using a video camera control unit.

### Indices of renal damage (glomerulosclerosis, tubulointerstitial and vascular damage)

The degree of sclerosis within the glomerular tuft as an index of progression (GSI) was determined on PAS-stained paraffin sections adopting the semiquantitative scoring system proposed by El Nahas et al. (1997). Using light microscopy at a magnification of x 400, the glomerular score of each animal was derived as the mean of 100 glomeruli. The severity of glomerulosclerosis was expressed on an arbitrary scale from 0 to 4 as described elsewhere.

Tubulointerstitial (TBI) and vascular damage (VDI) were assessed on PAS-stained paraffin sections at a magnification of x100 using a similar scoring system

with scores 0-4.

### Glomerular geometry

Area ( $A_A$ ) and volume density ( $V_V$ ) of the renal cortex and medulla, as well as the number of intact glomeruli per area ( $N_A$ ) were measured using a Zeiss eyepiece (Integrationsplatte II; Zeiss Co., Oberkochen, Germany) and the point counting method ( $PP = AA = VV$ ) at a magnification of x400, described in detail (Gross et al., 2004).

### Immunohistochemistry (Table 1)

Immunohistochemical stainings were performed on decalcified bone. Paraffin embedded bone sections were de-paraffinized and subjected to heat-induced antigen retrieval using a citrate buffer (Dako, Hamburg, Germany).

The following antibodies were used for immunohistochemistry: TGF- $\beta$  (Santa Cruz, Heidelberg, Germany), Osteoprotegerin (Santa Cruz, Heidelberg, Germany), Parathyroid Hormone / Parathyroid Hormone Related Peptide Receptor (PTH/PTHrP) (USBiological, Swampscott, USA), Growth hormone receptor (ABCAM for Biozol, Eching, Germany); IGF-1 (Santa Cruz, Heidelberg, Germany).

Immunohistochemical evaluation was performed independently by two pathologists using a semi-quantitative immunoreactive score: 0-12. The score is the product of the semi-quantitative scores for the intensity of the immunostaining (0 = negative; 1 = poor intensity; 2 = moderate intensity; 3 = severe intensity) and the semi-quantitative score for percentage of positive stained cells (0 = negative; 1 = <10%; 2 = 10-50%; 3 = 51-80%; 4 => 80%). Expression of the investigated proteins was analyzed in osteoblasts, osteoclasts, osteocytes and chondrocytes separately.

### Statistical analysis

All data are presented as mean  $\pm$  SD. Differences between groups were analyzed by one-way ANOVA followed by Duncan's test. P value less than 0.05 was

**Table 1.** Antibodies used for immunohistochemistry.

Marker	Primary antibody	Dilution	Secondary antibody	Detection system
Growth hormone receptor	Mouse monoclonal IgG1 [MAB 263]	1:50 in 2% goat serum	Horse Antimouse biotinylated (Rat adsorbed) (Biocare Medical)	ABC-Method
TGF- $\beta$	Rabbit polyclonal IgG1	1:100 in 2% goat serum	Goat antirabbit biotinylated (BioGenex)	ABC-Method
IGF-1	Goat polyclonal IgG1	1:25 in universal antibody diluent (Dako, Hamburg, Germany)	Mouse antigoat biotinylated 1:500 in universal antibody diluent (Jackson Immunoresearch)	ABC-Method
OPG	Rabbit polyclonal IgG1	1:10 in 1% goat serum	Goat antirabbit biotinylated (BioGenex)	ABC-Method
PTHrP	Mouse monoclonal IgG1	1:20 in universal antibody diluent (Dako, Hamburg, Germany)	Horse Antimouse biotinylated (Rat adsorbed) (Biocare Medical)	ABC-Method

accepted as being statistically significant. All statistical analysis was done with Statistica 5.0 software (StatSoft, Inc. Tulsa, USA).

**Results**

*Animal data (Table 2)*

Body weight was significantly reduced after 10 weeks of CRF. Treatment with GH improved the body weight irrespective of the dose given, but only the group treated with the highest dose reached a final body weight that did not differ significantly from the weight of the sham-operated animals. All SNX groups, as compared to the sham group, exhibited increased blood pressure levels, but no differences were observed between the GH treated and vehicle treated SNX groups. The creatinine concentration increased in all animals after SNX, and though on average higher in GH treated group, the levels were not significantly different from the vehicle treated SNX group and did not correlate with the GH dose administered.

*Histology of femoral bones and the kidneys (fig.1, 2, table 3 and 4)*

**Bone**

Vehicle treated SNX animals showed a marked reduction of bone tissue: trabecular bone volume, cortical bone volume, osteoid volume, resorptive surface, mean cortical width as well as trabecular thickness were significantly lower in this group compared to control animals (p<0.05). In contrast, the number of osteoclasts and the resorptive surface were significantly higher in SNX animals versus control animals.

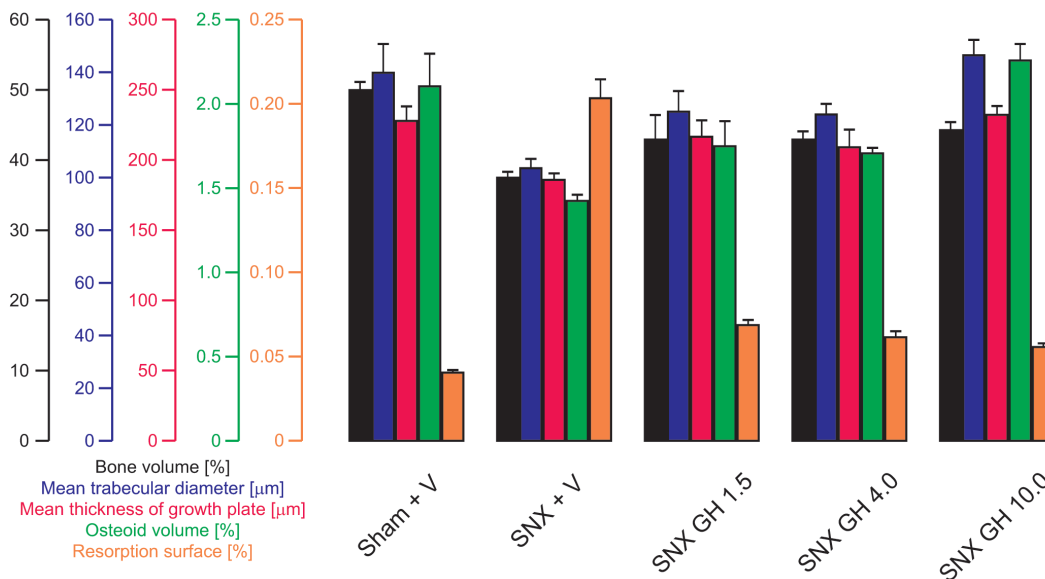
All animals treated with GH showed a positive dynamic of bone synthesis. GH treatment resulted in a decrease of resorption area and number of osteoclasts, as well as an increase of bone and osteoid volume, number of osteoblasts, percentage of active osteoblasts, thickness of the growth plate and mean cortical width.

The effect of GH treatment on bone volume was independent from administrated GH dose. All animal

**Table 2.** Basic animal data.

	Sham+V	SNX+V	SNX+GH 1.5	SNX+GH 4.0	SNX+GH 10.0	ANOVA
Body weight (study beginning) [g]	148±8	149±6	150±9	153±12	146±10	NS
Body weight at GH/V (treatment start) [g]	508±35	409±21*	406±50*	422±21*	407±63*	p<0.001
Body weight (study end) [g]	574±33	424±26*	482±45*	487±57*	506±62	p<0.001
Body weight increase during treatment [g]	66.0±14.6#	15.2±6.7*	76.0±23.3#	65.6±45.3#	99.1±39.0#	p=0.002
Creatinine concentration (study end) [mg/dl]	0.33±0.10	0.62±0.0.11*	0.82±0.15*	1.02±0.31*	0.93±0.22*	p<0.001
Blood pressure (study end) [mmHg]	119.7±5.1	148.8±15.9*	140.6±8.3*	140.3±16.6*	144.6±9.8*	p=0.001

\*: p<0.05 vs. Sham+V; #: p<0.05 vs. SNX+V



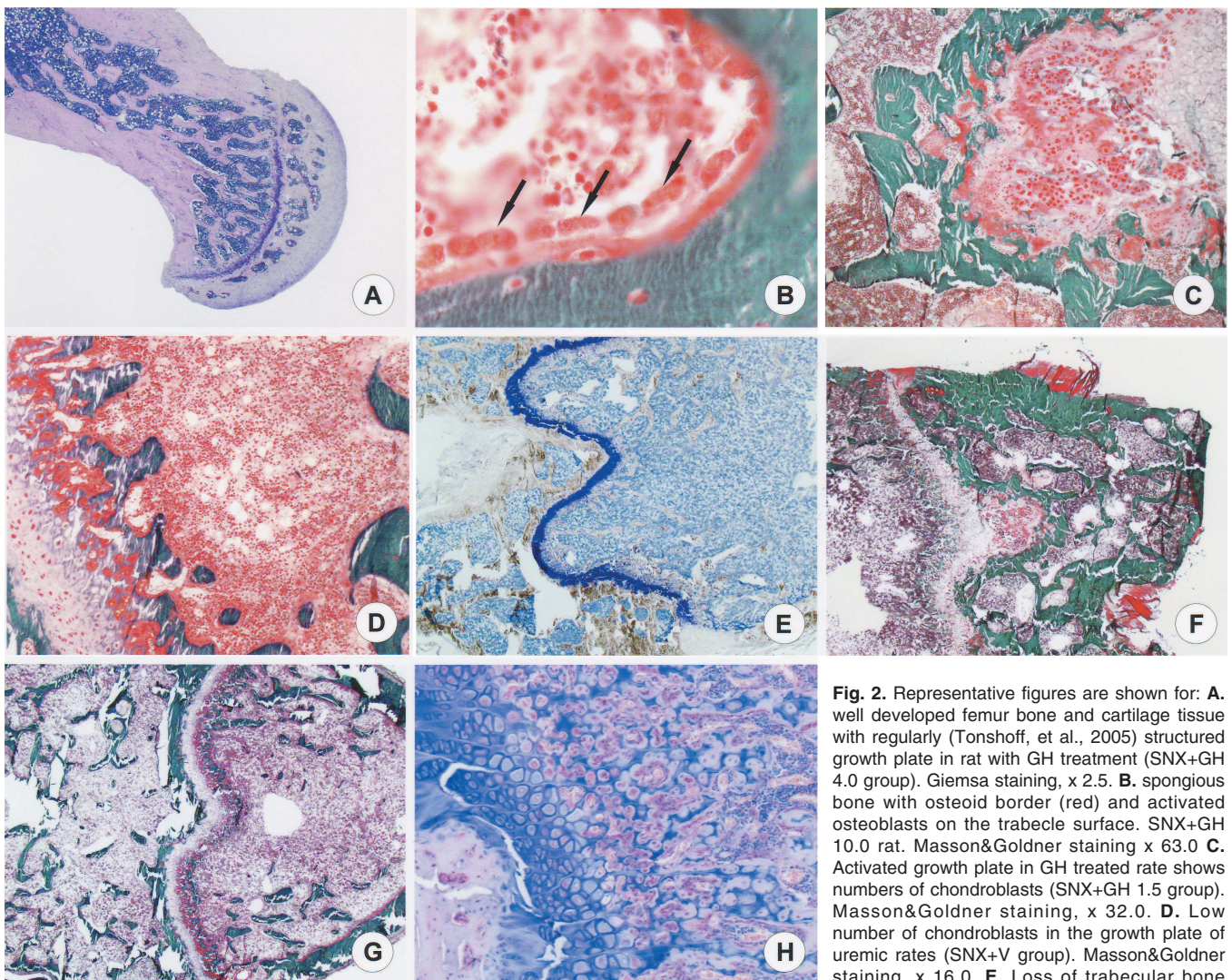
**Fig. 1.** Dynamic of bone metabolism in investigated animal groups.

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**Table 3.** Morphological bone data.

	Sham+V	SNX+V	SNX+GH 1.5	SNX+GH 4.0	SNX+GH 10.0	ANOVA
Bone volume [%]	49.99±1.31	37.81±0.64*	43.20±3.63*#	43.21±1.22*#	44.65±0.99*#	p<0.001
Mean trabecular diameter [µm]	139.5±11.9	103.2±3.4*	125.1±7.4*#	123.6±4.0#	145.9±6.3#\$\$	p<0.001
Mean corticalis width [µm]	821.9±25.0	717.7±27.2*	718.5±22.3*	757.8±18.8*#	734.2±30.6*#	p<0.001
Mean thickness of growth plate [µm]	227.3±11.0	186.6±4.4*	217.1±11.9	209.6±12.3	232.8±6.8#	p<0.005
Osteoblasts surface [%]	54.14±1.81	42.54±1.89*	48.14±0.28*#	48.57±0.90*#	52.26±1.39#\$\$	p<0.001
Percentage of active osteoblasts [%]	49.49±1.15	35.34±2.48*	43.32±1.04*#	43.71±0.99#	51.29±1.95#\$\$	p<0.001
Osteoid volume [%]	2.10±0.18	1.42±0.02*	1.75±0.14*	1.70±0.03#	2.25±0.10#§	p<0.005
Number of osteoclasts [per 1/mm <sup>2</sup> of bone trabecle ]	2.01±0.03	8.09±0.56*	3.98±0.15#	3.79±0.21#	3.80±0.20#	p<0.001
Resorption surface [%]	0.040±0.001	0.203±0.012*	0.068±0.003#	0.061±0.004#	0.055±0.002#	p<0.005

\*: p<0.05 vs. Sham+V; #: p<0.05 vs. SNX+V; §: p<0.05 vs. SNX+GH 1.5; \$: p<0.05 vs. SNX+GH 4.0.



**Fig. 2.** Representative figures are shown for: **A.** well developed femur bone and cartilage tissue with regularly (Tonshoff, et al., 2005) structured growth plate in rat with GH treatment (SNX+GH 4.0 group). Giemsa staining, x 2.5. **B.** spongy bone with osteoid border (red) and activated osteoblasts on the trabecle surface. SNX+GH 10.0 rat. Masson&Goldner staining, x 63.0. **C.** Activated growth plate in GH treated rat shows numbers of chondroblasts (SNX+GH 1.5 group). Masson&Goldner staining, x 32.0. **D.** Low number of chondroblasts in the growth plate of uremic rates (SNX+V group). Masson&Goldner staining, x 16.0. **E.** Loss of trabecular bone tissue in uremic rats (SNX+V group). Giemsa staining, x 8.0. **F.** High density of trabecular bone in GH treated animals (SNX+GH 4.0 group). Masson&Goldner staining, x 6.0. **G.** Loss of primary bone trabecle in uremic rats (SNX+V group). Giemsa staining, x 6.0. **H.** positive cytoplasmic staining for GH in chondrocytes, osteoblasts and osteoclasts of GH treated animals (SNX+GH 10.0 group). Immunohistochemical staining for GH, x 40.

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groups with GH treatment showed statistically significant increases of the bone volume in comparison with vehicle treated SNX animals ( $p < 0.05$ ). The differences between GH treated groups regarding bone volume were statistically not significant. In spite of an increase of bone volume in GH treated animals, this parameter remained lower in comparison with the control group ( $p < 0.05$ ).

The thickness of the proximal femur growth plate showed significant differences between GH and vehicle treated SNX rats and was  $227.27 \pm 10.96$ ,  $186.56 \pm 4.36$ ,  $217.05 \pm 11.90$ ,  $209.64 \pm 12.34$  and  $232.84 \pm 6.84 \mu\text{m}$  for the Sham, SNX+V, SNX+GH 1.5, SNX+GH 4.0 and SNX+GH 10.0 groups, respectively (see table 3). The differences between vehicle treated SNX rats and SNX+GH 10.0 treated rats were statistically significant ( $p < 0.05$ ).

A similar tendency was seen by analysis of osteoid volume. An increase of osteoid volume was detected in all GH treated groups versus vehicle treated SNX rats and was maximal in SNX+GH 10.0 treated rats ( $p < 0.05$ ).

Statistically significant increases of the trabecular bone volume, mean cortical width, trabecular thickness and thickness of the growth plate were observed in all animal groups with the GH treatment. The effect was dependent on GH dose and was maximal in the group with GH treatment 10 mg/kg SC.

GH treatment leads to a statistically significant reduction of the number of osteoclasts, which was increased in the SNX-V group, and a simultaneous decrease in the resorptive surface. No significant differences between GH treated groups were found.

#### Kidney (see Table 5)

Uremia was associated with a significantly increased glomerulosclerosis (GSI), tubulointerstitial damage index (TBI), low number of glomeruli and elevated mean glomerular volume. The glomerulosclerosis (GSI), and tubulointerstitial (TII), damage indices were significantly higher in both untreated and treated SNX groups than in sham-operated controls. In contrast to the beneficial effect of GH treatment on bone, GH had a

**Table 4.** Immunohistochemical staining.

staining / cell type	Sham+V	SNX+V	SNX+GH 1.5	SNX+GH 4.0	SNX+GH 10.0	ANOVA
GHR						
osteoblasts	7.33±2.25	2.60±1.34*	5.33±1.86	5.57±1.90#	8.00±3.63#	p<0.01
osteoclasts	6.33±3.01	2.80±1.30*	4.17±1.83	6.57±1.90	5.67±2.34	p<0.05
chondroblasts	6.17±2.04	2.00±1.58*	2.00±1.87*	2.29±1.25*	5.67±2.88#§§	p=0.020
IGF-1						
osteoblasts	5.80±1.79	2.75±1.26*	6.71±1.70#	5.57±1.81#	3.14±1.77*\$	p<0.005
osteoclasts	2.80±0.84	4.50±1.29*	5.14±0.38*	4.00±1.41*	3.00±0.58#§	p<0.001
chondroblasts	9.40±1.82	1.75±0.50*	6.00±1.63*#	5.14±1.07*#	4.29±1.25*#	p<0.001
TGF-β1						
osteoblasts	2.86±0.69	6.00±0.71*	2.86±0.90#	4.57±0.53*#	5.43±1.13*	p<0.001
osteoclasts	2.71±1.11	5.00±1.41*	2.86±0.38#	3.71±0.95	4.57±1.40*	p=0.002
chondroblasts	2.29±1.25	0,294±0,21	1.00±1.29	2.43±2.51	2.57±3.21	p=0.2
PTHrPR						
osteoblasts	3.50±0.84	2.20±1.30	6.00±1.41#	5.14±1.46#	2.33±0.98	p<0.001
osteoclasts	1.33±1.03	3.00±1.22*	5.57±2.37*#	4.86±1.35*#	2.83±0.98	p<0.001
chondroblasts	4.83±0.98	2.40±1.82*	5.86±1.46#	6.29±2.63#	1.50±1.52*	p<0.001
OPG						
osteoblasts	0.14±0.38	1.00±1.00	0.43±0.79	1.71±1.11*	1.86±1.35*	p=0.009
osteoclasts	2.29±0.49	2.00±0.00	2.29±0.49	3.14±1.35	4.71±2.43*#	p=0.005
chondroblasts	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NS

\*: p<0.05 vs. Sham+V; #: p<0.05 vs. SNX+V; \$: p<0.05 vs. SNX+GH 1.5; §: p<0.05 vs. SNX+GH 4.0.

**Table 5.** Morphological data of the kidney.

	Sham+V	SNX+V	SNX+GH 1.5	SNX+GH 4.0	SNX+GH 10.0	ANOVA
Kidney to body weight ratio [g/100g]	0.36±0.02	0.58±0.10*	0.57±0.17*	0.67±0.29*	0.57±0.12*	p=0.030
Number of glomeruli [1/mm <sup>3</sup> ]	17.92±2.77	4.44±1.51*	4.18±0.99*	3.34±1.38*	4.17±1.00*	p<0.001
Mean glomerular volume [10-6μm <sup>3</sup> ]	3.15±0.60	9.14±2.68*	9.12±1.13*	13.16±3.41*#	8.94±2.27*	p<0.001
GSI [score 0-4]	0.79±1.19	2.12±0.54*	2.29±0.59*	2.97±0.23*	2.66±0.85*	p<0.001
TII [score 0-4]	0.58±0.44	3.13±0.41*	3.77±0.22*#	3.78±0.20*#	3.79±0.27*#	p<0.001

\*: p<0.05 vs. Sham+V; #: p<0.05 vs. SNX+V.

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negative impact on the damaged kidney: TBI worsened and the glomerular volume increased.

### Immunohistochemistry (see Table 4)

GH receptor (GHR) protein expression was down regulated in osteoblasts, osteoclasts and chondroblasts in vehicle treated uremic animals in comparison with control animals ( $p < 0.05$ ). All GH-treated animal groups showed a statistically significant increase of GHR in osteoclasts and osteoblasts. The effect was proportional to the administered dose and was maximal in the SNX+GH 10.0 treated rats. In contrast to this, a statistically significant increase of GHR staining was seen in chondroblasts only in the SNX+GH 10.0 group. GHR staining on osteocytes did not show any relevant differences between investigated groups.

The TGF- $\beta$  expression was down regulated in SNX+V group in both osteocytes ( $p < 0.05$ ) and chondroblasts ( $p < 0.05$ ) compared to sham operated animals. The down regulation was prevented in GH treated animals irrespective of the dose given (s. Table 4). Osteoclasts and osteoblasts showed an increased expression of TGF- $\beta$  in uremic condition (SNX+V rats). In SNX+GH 1.5 rats a significant decrease of TGF- $\beta$  staining was found in both osteoclasts ( $p < 0.05$ ) and osteoblasts ( $p < 0.05$ ) in comparison with SNX+V rats. These parameters were similar to those in control animals. In contrast to this, SNX+GH 4.0 and SNX+GH 10.0 rats versus SNX+GH 1.5 rats showed an increased expression of TGF- $\beta$  in osteoclasts ( $p < 0.05$ ) and osteoblasts ( $p < 0.05$ ), but not in chondroblasts ( $p = 0.02$ ).

Expression of IGF was increased in osteoclasts of SNX+V rats versus control animals. This tendency was unvaried in SNX+GH 1.5 and SNX+GH 4.0 groups while in the SNX+GH 10.0 animals IGF-1 expression decreased to control values. In contrast to the osteoclasts, IGF-1 expression was decreased in osteoblasts and chondroblasts of SNX-V rats and increased significantly following GH treatment.

No staining for OPG was found in chondroblasts in investigated animals.

Expression of PTHRPR was increased in osteoclasts of SNX+V rats versus control animals. In contrast to this finding, expression of PTHRPR was decreased in osteoblasts and chondroblasts of SNX+V rats compared with control group. GH treatment in the SNX+GH 1.5 rats led to a significant increase of PTHRPR expression in all investigated cells. An increased dose of GH resulted in progressive decrease of PTHRPR expression in these cells. Expression of PTHRPR was low in chondroblasts and osteoblasts in the SNX+GH 10.0 rats versus Sham+V, SNX+V, SNX+GH 1.5 and SNX+GH 4.0 animals. Expression of PTHRPR in osteoclasts was lowest in the SNX+GH 10.0 animals in the GH treated animal groups, but was higher compared with control.

### Discussion

The normal function of bone tissue depends strongly

on its mineralization. This process is highly altered in uremia and contributes to a failure of bone growth and bone turn over.

Recombinant growth hormone is widely used for the treatment of growth retardation accompanying uremia.

In this study we analyzed the effect of GH treatment over a wide range of doses on bone structure in mature uremic rats. Despite a negative effect on renal structure, a positive impact of GH treatment on bone turn over was detected. GH treatment increased the thickness of the growth plate and bone mass by both stimulating osteoblastic activity and inhibiting bone resorption in uremic animals. We observed a significant decrease in osteoblast and chondroblast IGF-1 expression in untreated uremic rats compared to control animals, which increased following GH treatment: GHR levels were also reduced in uremia and increased following GH therapy. These results correspond to previously published data regarding the expression of the GH receptor in the growth plate in rats with chronic renal failure and the response to GH treatment. Edmondson et al. reported a decreased expression of GHR and IGF-1 in the proliferative zone of uremic rats as well as reduced chondrocyte proliferation, and showed that growth hormone treatment improves chondrocyte maturation and activates bone metabolism in the primary spongiosa (Edmondson et al., 2000). Immunohistochemical staining for the GH receptor reveals its low abundance in growth plate chondrocytes of uremic animals, and this was reversed with GH and IGF-I treatment (Edmondson, et al., 2000). In addition to these findings we observed that GH treatment had a significant positive impact on bone structure; there was a significant increase in number of osteoblasts combined with an increase of osteoid volume and trabecular width compared to the vehicle treated uremic rats (Fig. 1).

On the other hand, GH treatment reduced bone resorption by decreasing the number of osteoclasts and lowering the functional activity of these cells, which resulted in a remarkable decrease of bone surface. These parameters were significantly altered over a wide range of GH doses and were similar to those in control animals. Nishiyama et al. described a stimulatory effect of GH in bone resorption and osteoclast differentiation in vitro (Nishiyama et al., 1996). Furthermore, the authors reported that GH had a positive impact on the activation of quiescent osteoclasts as well as the development of new osteoclasts by stimulation of osteoclast-like cell formation from hemopoietic blast cells. However, the exact mechanism of the osteoclast activation by GH treatment remains unclear. Corresponding with these data, we found a higher number of osteoclasts and increased resorptive surface in GH treated uremic rats versus control animals. In contrast to these results, we observed a relevant reduction of osteoclast number in GH treatment compared to V treated uremic rats. However, the underlying mechanisms of this phenomenon are not completely understood and should be investigated in further studies.

GH has direct effects on chondrocytes, but primarily it regulates osteoblasts and chondroblasts function through IGF-1 by stimulation of the proliferation and matrix production in these cells (Tonshoff et al., 2005). We observed a significant decrease of IGF-1 expression in chondroblasts and osteoblasts of untreated uremic rats compared to control animals. This is consistent with the reduction in GH receptor expression, possibly caused by a defect in post receptor signal transduction through the JAK2/STAT5 pathway (Rabkin et al., 2005). Treatment with recombinant GH in relatively high doses was able to overcome the resistant state and increased IGF-1 protein expression. This accounts for the beneficial effects of GH treatment on bone growth and turn over.

The exact mechanism of the salutary effect of GH on bone is not fully understood because of controversial data about the impact of GH on chondromodulin-1 metabolism, proteins related to matrix degradation and control of angiogenesis. However, it is possible that in addition to increasing circulating IGF-1 levels, GH may directly increase local IGF-1 levels and thus maintain cartilage and bone growth. In an *in vivo* model, IGF-1 infusion for 7-14 days stimulated an increase of trabecular and cortical bone densities (Spencer et al., 1991). However, the effect of GH on bone resorption in cell cultures seems to be also stimulatory (Nishiyama et al., 1996). It has to be considered that these studies were not performed in uremic conditions, where the bone resorption is significantly increased prior to the treatment.

We observed a significantly increased expression of the IGF-1 in osteoclasts of uremic rats versus control animals, and GH treatment did not lead to changes of IGF-1 expression in these cells. At the same time, both resorptive surface and osteoclast number were reduced in rats with GH treatment versus V treated uremic animals. This phenomenon seems to be controversial in comparison to previously published *in vitro* studies which demonstrated a stimulatory effect of GH on the osteoclast differentiation and activity (Olney, 2003).

Previous pharmacologic, genetic and clinical studies have shown that IGF-1, rather than GH is the major hormone that directly induces bone growth (Roelfsema and Clark, 2001). However, it has been demonstrated that for some responses the presence of both hormones are required. Thus it is debatable whether the effect of GH on bone cells that we have described in the uremic rat is solely due to the observed increase in IGF-1.

Another postulated mechanism of GH action on bone remodeling is its impact on PTH sensitivity. In the study of White et al. PTH sensitivity was reduced in older GH deficient patients, and following GH treatment, PTH sensitivity was increased and was accompanied by an increase in bone turnover markers (White et al., 2005).

Previous studies demonstrated a down regulation of the expression of PTH/PTHrP receptor in osteoblasts in uremia (Picton et al., 2000). Our results correspond to published data and show additionally a low expression

of PTHRPR in chondroblasts of uremic mice lacking GH treatment. In contrast to this, expression of PTHRPR in osteoclasts was significantly higher in uremic rats versus control animals. An elevated expression of PTHRPR in these cells in uremic rats was accompanied by an increased number of osteoclasts as well as an increase of bone resorption. GH treatment led to an elevation of the PTHRPR in all investigated cells. This phenomenon was accompanied by an activation of the bone turn over. GH treatment is able to influence the PTH/PTHrP receptor expression *in vivo* (Urena et al., 1996; Sanchez and He, 2004). In cell culture studies, PTH affected both bone formation and bone resorption, suggesting that the net result of PTH therapy may be either bone gain or bone loss, depending on the dosage, mode of administration, bone site, and animal species (Thomas, 2006). Corresponding to these data, histological studies established that intermittent PTH therapy was associated with an increase in trabecular bone and, importantly, with improvements in trabecular and cortical microarchitectural parameters that have not been reported with antiresorptive drugs (Sato et al., 2002). Based on published and our own results, we assume the anabolic effect of GH treatment by additional modulation of PTHRPR expression in uremic rates. However, this effect seems to be dependent on GH dose. Maximal GH dose (SNX+GH 10.0 animals) led to a decrease of the PTHRPR expression in all investigated cells, especially in osteoblasts and chondroblasts. Although we did not observe a decrease in the number of cells, we registered a decreased function of osteoclasts corresponding with a decrease of resorptive surface of the bone.

It should be noted that while GH therapy did not alter the serum creatinine levels significantly in our study animals, it caused an increase in tubulointestinal damage. Several reports have implicated GH in the development and progression of kidney disease. For example, GH transgenic mice develop glomerulosclerosis (Doi et al., 1988) and GH therapy accelerates the progression of kidney disease in rodents (Allen et al., 1992; Trachtman et al., 1993). However the adverse renal effect of pharmacologic doses of GH appears to be restricted to rodents, because extended GH treatment has no detrimental effects on the kidney disease progression in children with CRF (Tonshoff et al., 1992; Haffner et al., 2000).

In conclusion, we observed that administration of recombinant GH to rats with chronic renal failure and with reduced bone mass has a positive effect on bone turn over, increasing the reduced bone volume. Since this occurred without any change in renal function as judged by serum creatinine levels, we conclude that the positive dynamic of bone turn over is due to GH treatment only, and is not mediated by any improvement of the kidney function. Finally, our results indicate that in mature uremic rats, bone tissue is responsive to the administration of recombinant GH, and that this intervention may conceivably be of value in the



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management of patients with renal osteoporosis.

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