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Insulin-like growth factor system components in hyperparathyroidism and renal osteodystrophy

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Insulin-like growth factor system components in hyperparathyroidism and renal osteodystrophy.

Background. The insulin-like growth factor (IGF) system plays a key role in regulation of bone formation. In patients with renal osteodystrophy, an elevation of some IGF binding proteins (IGFBPs) has been described, but there is no study measuring serum levels of both IGF-I and IGF-II as well as IGFBP-1 to -6 in different forms of renal osteodystrophy and hyperparathyroidism.

Methods. In a cross-sectional study, we investigated 319 patients with mild ($N = 29$), moderate ($N = 48$), preuremic ($N = 37$), and end-stage renal failure (ESRF; $N = 205$). The ESRF group was treated by hemodialysis (HD; $N = 148$), peritoneal dialysis (PD; $N = 27$), or renal transplantation (RTX; $N = 30$). As controls without renal failure, we recruited age-matched healthy subjects ($N = 87$) and patients with primary hyperparathyroidism (pHPT; $N = 25$). Serum levels of total and free IGF-I, IGF-II, IGFBP-1 to -6, and biochemical bone markers including intact parathyroid hormone (PTH), bone alkaline phosphatase (B-ALP), and osteocalcin (OSC) were measured by specific immunometric assays. IGF system components and bone markers were correlated with clinical and bone histologic findings. Mean values ± SEM are given.

Results. With declining renal function a significant increase was measured for IGFBP-1 (range 7- to 14-fold), IGFBP-2 (3- to 8-fold), IGFBP-3 (1.5- to 3-fold), IGFBP-4 (3- to 19-fold), and IGFBP-6 (8- to 25-fold), whereas IGFBP-5 levels tended to decrease (1.3- to 1.6-fold). In contrast, serum levels of IGF-I, free IGF-I, and IGF-II remained constant in most patients. Compared with renal failure patients, pHPT patients showed a similar decline in IGFBP-5 levels and less elevated levels of IGFBP-1 (3.5-fold), IGFBP-2 (2-fold), IGFBP-3 (1.2-fold), and IGFBP-6 (4-fold) but no elevation of IGFBP-4 levels. In all subjects, free and total IGF-I levels showed significant negative correlations with IGFBP-1, IGFBP-2, and IGFBP-4 (that is, inhibitory IGF system components) and significant positive correlations with IGFBP-3 and IGFBP-5 (that is, stimulatory IGF system components). A positive correlation was observed between IGF-II and IGFBP-6. ESRF patients with mixed uremic bone disease and histologic evidence for osteopenia revealed significantly ($P < 0.05$) higher levels of IGFBP-2 and IGFBP-4 but lower IGFBP-5 levels. Histologic parameters of bone formation showed significant positive correlations with serum levels of IGF-I, IGF-II, and IGFBP-5. In contrast, IGFBP-2 and IGFBP-4 correlated positively with indices of bone loss. Moreover, dialysis patients with low bone turnover ($N = 24$) showed significantly ($P < 0.05$) lower levels of IGFBP-5, PTH, B-ALP, and OSC than patients with high bone turnover.

Conclusion. Patients with primary and secondary hyperparathyroidism showed lower levels of the putative stimulatory IGFBP-5 but higher levels of IGFBP-1, -2, -3, and -6, whereas total IGF-I and IGF-II levels were not or only moderately increased. The marked increase in serum levels of IGFBP-4 appeared to be characteristic for chronic renal failure. IGFBP-5 correlated with biochemical markers and histologic indices of bone formation in renal osteodystrophy patients and was not influenced by renal function. Therefore, IGFBP-5 may gain significance as a serological marker for osteopenia and low bone turnover in long-term dialysis patients.

Key words: osteopenia, low bone turnover, dialysis, uremic bone disease, end-stage renal failure.

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Bone remodeling is regulated by systemic hormones and locally produced factors acting in concert to maintain bone mass [1, 2]. Insulin-like growth factors (IGFs) are among the most important regulators of bone cell function because of their abundance and their proven anabolic effects on the skeleton (decreasing collagen degradation, increasing bone matrix deposition, and increasing osteoblastic cell recruitment) [3, 4]. The key role of the IGF system in the local regulation of bone formation is demonstrated by the finding that approximately 50% of basal bone cell proliferation could be blocked by inhibiting the actions of IGF-I and IGF-II endogenously produced by bone cells in serum-free cultures [4]. IGF-I is

known to be the mediator of growth hormone action in various tissues, including bone, whereas the biological role of circulating IGF-II is unclear. Most of the circulating IGFs (≥99%) are bound to six specific high-affinity IGF binding proteins (IGFBPs), which modulate IGF action both in a positive and negative manner [5–7]. IGFBP-3, the quantitatively predominant IGFBP in circulation, is positively regulated by growth hormone and potentiates IGF action under most conditions. IGFBP-3 has the unique property of being able to associate with an acid-labile subunit (ALS) after IGF binding, thus forming a 150 kd complex. This large complex raises the plasma half-life of IGFs from few minutes to several hours, as this complex is essentially limited to the intravascular space. On the other hand, 20 to 30% of the serum IGFs are found in small (45 kd) protein complexes that contain IGFBPs but not ALS. The low molecular weight IGF-IGFBP complexes can leave the intravascular space and are supposed to be the primary vehicle that allows circulating IGFs to reach extravascular tissue binding sites [8]. IGFs are often coexpressed with IGFBPs in various tissues (for example, liver, kidney, bone), and circulating as well as locally expressed IGFBPs appear to modify IGF action by either inhibiting or promoting IGF bioactivity. In bone cells, locally produced IGF system components may act as potential endogenous mediators of a variety of osteoregulatory agents, including PTH and calcitriol, and may modulate IGF actions in both a positive (for example, IGFBP-5) and negative manner (for example, IGFBP-4) [3, 4, 9, 10].

In contrast to the well-defined local action of IGF-I on the skeleton, the relationship between circulating IGF system components and bone remodeling is less well understood. PTH infusion increased circulating levels of IGFBP-3 in healthy women but not in patients with inflammatory active rheumatoid arthritis. However, when inflammation had subsided, the patients responded with a similar increase in IGFBP-3 levels as the control group [11]. In patients with idiopathic osteoporosis, decreased serum levels of IGFBP-3 have been reported [12]. Another study showed that IGF-I serum levels showed a significant positive correlation with the osteoblastic surface measured by histomorphometric techniques [13]. In insulin-dependent diabetes mellitus (IDDM) patients, it has been shown that low IGF-I and elevated IGFBP-1 levels contribute to impaired growth in prepubertal subjects [14]. In IDDM patients, we recently observed that bone mineral density was positively correlated with stimulatory IGF system components (IGF-I, IGFBP-3, and IGFBP-5) and negatively correlated with inhibitory IGF system components (IGFBP-1 and IGFBP-4) [15]. In older women with hip and spine fractures, increased IGFBP-4 serum levels have been reported, which were positively correlated to elevated PTH concentrations [16, 17]. Although circulating IGF system components may only be partially influenced by bone cell production, these studies suggest that circulating levels of IGF-I and IGFBPs are significantly related to bone metabolism and thus may be used as bone markers.

Although in the last two decades great progress has been made concerning renal replacement therapy, renal osteodystrophy is still of major clinical importance in patients with chronic renal failure (CRF). When renal function declines by 50%, the kidneys are no longer able to synthesize appropriate amounts of active metabolites of vitamin D_3, thus leading to the development of secondary hyperparathyroidism (sHPT) [18, 19]. In sHPT, bone histologic findings include an increased bone resorption caused by enhanced osteoclastic activity, as well as osteoidosis and osteomalacia. For the diagnosis and differential therapy of the different forms of renal osteodystrophy, bone biopsy and biochemical markers of bone metabolism are well-established tools [20, 21]. In this cross-sectional study, we tested the hypothesis of whether the measurement of circulating IGF system components as putative anabolic regulators of bone metabolism may shed new light on the complex pathophysiology of different forms of hyperparathyroidism and renal osteodystrophy. Our data clearly indicate an imbalance between serum levels of IGFs and IGFBPs with declining renal function that may lead to a lower bioavailability of IGF-I to target tissues such as bone resulting in impairment of bone formation. Concerning the IGF system components measured, in renal osteodystrophy, elevated IGFBP-4 and decreased IGFBP-5 levels may be particularly associated with osteopenia and low bone turnover (LTO).

METHODS

Patients

In this cross-sectional study, we investigated 319 patients with different stages of CRF from three hospitals and four dialysis centers. As controls without renal failure, we recruited age-matched healthy subjects ($N = 87$) and patients with primary hyperparathyroidism (pHPT; $N = 25$). The study protocol was approved by the local ethics committee. All subjects gave informed consent to participate in this study. Clinical and laboratory findings are given in Table 1. The CRF group consisted of 29 subjects with mild, 48 with moderate, 37 with preuremic, and 205 with end-stage renal failure (ESRF). The cause of renal failure included chronic glomerulonephritis ($N = 93$, 29%); interstitial nephropathy, including chronic pyelonephritis, obstructive uropathy, and vesicoureteral reflux ($N = 54$, 17%); hereditary nephropathy, including polycystic kidney disease ($N = 44$, 14%); diabetic nephropathy ($N = 44$, 14%); renovascular disease ($N = 30$, 9%); analgesic nephropathy ($N = 10$, 3%);

Table 1. Clinical data and laboratory findings (reference values in parentheses) in patients with primary hyperparathyroidism (pHPT) and different stages of chronic renal failure (CRF)

N	pHPT 25	CRF I° 29	CRF II° 48	CRF III° 37	ESRF/HD 148	ESRF/PD 27	ESRF/RTX 30 (Σ = 344)
Sex *female/male*	17/8	17/12	20/28	10/27	68/80	13/14	10/20
Age *years*	60 ± 3 [23–83]	56 ± 2.6 [21–79]	56 ± 2.6 [26–82]	57 ± 2.5 [20–82]	56 ± 3 [17–84]	45 ± 3[a] [21–75]	47 ± 2[a] [24–66]
Duration of CRF *years*	—	3.1 ± 0.6 [0.3–13]	5.9 ± 1.2 [0.2–25]	6.9 ± 0.8 [0.2–21]	7.4 ± 0.6 [0.1–37]	9.4 ± 1[b] [1–33]	12.3 ± 1.2[bc] [3–34]
Duration of HD, PD, or RTX *years*	—	—	—	—	4.4 ± 0.4 [0.1–20]	5.2 ± 0.5 [1–13]	7.0 ± 0.6[c] [2–16]
Creatinine *58–110 μmol/L*	78 ± 5 [60–108]	160 ± 8[a] [111–245]	359 ± 11[a] [226–540]	675 ± 27[a] [488–1114]	692 ± 20[a] [327–1353]	898 ± 50[a] [680–1541]	263 ± 32[abc] [83–734]
Hemoglobin *12–15 g/dL*	14.4 ± 0.5 [12–15.8]	12.2 ± 0.6[a] [11–15.3]	11.4 ± 0.3[a] [7.8–15.6]	9.9 ± 0.3[a] [7.3–14.6]	9.8 ± 0.2[a] [7–14.5]	10.0 ± 0.2[a] [7.5–13.2]	12.1 ± 0.4[abc] [8.2–16.7]
PTH *11–54 pg/mL*	193 ± 35 [79–950]	142 ± 30 [14–627]	303 ± 40[a] [14–1365]	374 ± 37[a] [69–1135]	345 ± 30[a] [2–1470]	309 ± 57[a] [6–1174]	273 ± 45[ab] [21–1160]
B-ALP *7–17 ng/mL*	20.7 ± 4 [4–68]	27.4 ± 11 [3–38]	20.6 ± 3 [3–63]	15.3 ± 2 [6–53]	23.7 ± 4 [4–353]	16.9 ± 2 [5–51]	19.3 ± 2 [5–424]
OSC *11–48 ng/mL*	39 ± 6 [7–135]	40 ± 7 [4–115]	66 ± 13[a] [7–422]	161 ± 34[a] [8–958]	185 ± 39[a] [11–2280]	186 ± 71[a] [13–1602]	137 ± 71[a] [7–2244]
PICP *40–200 ng/mL*	157 ± 16 [55–439]	173 ± 23 [81–531]	213 ± 22[a] [53–660]	211 ± 25[a] [66–675]	224 ± 15[a] [66–1066]	300 ± 28[a] [76–587]	265 ± 58[a] [88–529]
25-D_3 *10–60 ng/mL*	18.7 ± 2 [6–41]	18.3 ± 3 [2–50]	24.4 ± 4 [5–49]	27.6 ± 6 [4–52]	30.3 ± 5[a] [4–63]	15.6 ± 3 [5–25]	18.1 ± 2 [4–39]
1.25-D_3 *30–70 pg/mL*	78.4 ± 7 [22–138]	30.7 ± 5[a] [3–67]	17.5 ± 2[a] [5–31]	11.5 ± 1[a] [5–25]	12.9 ± 1[a] [0.6–50]	5.6 ± 2[ab] [3–12]	29.6 ± 5[abc] [1–56]

Data are mean values [range] ± SEM. Abbreviations are in the Appendix.
[a] $P < 0.05$ vs. pHPT
[b] $P < 0.05$ vs. CRF III°
[c] $P < 0.05$ vs. PD

multiple system disorders (N = 10, 3%); and unknown origin (N = 34, 11%).

The stages of CRF were defined as follows: CRF I°, mild renal insufficiency with creatinine up to 250 μmol/L and hyperparathyroidism but no further clinical signs of CRF; CRF II°, moderate renal insufficiency with serum creatinine levels between 250 and 500 μmol/L and additional signs of CRF such as incipient anemia or metabolic acidosis; CRF III°, serum creatinine levels above 500 μmol/L plus typical clinical manifestations of the complex uremic syndrome (for example, metabolic acidosis, fluid overload, electrolyte disturbances, accumulation of end-products of protein metabolism). With end-stage renal failure (ESRF), patients were on maintenance renal replacement therapy by bicarbonate hemodialysis (HD; N = 148), peritoneal dialysis (PD; N = 27), or renal transplantation (RTX; N = 30). Six HD patients had parathyroidectomy prior to the study.

In 28 HD patients, anterior iliac crest bone biopsies were performed under local anesthesia. Transcortical bone samples of 2 to 3 cm in length and 5 mm in diameter were obtained using an electric drill (Straumann, Cambridge, MA, USA). Histologic analysis of Goldner-stained sections was performed as previously described [19, 21]. Separate sections were also stained with aurintricarboxylic acid for the identification of aluminum on mineralizing bone surfaces. Bone biopsies were analyzed qualitatively and semiquantitatively. In each bone biopsy, the following parameters were assessed and graded according to a four-point semiquantitative scale (−2 = markedly decreased; −1 = decreased; 0 = normal, 1 = increased): cortical bone thickness, trabecular bone volume, trabecular thickness, trabecular interconnections, osteoid thickness, endosteal fibrosis, osteoblastic surface, osteoclastic surface, and bone marrow hematopoietic cell volume. All measurements were performed blindly by the same person without the knowledge of the corresponding serum levels of bone markers and IGF system components.

Hemodialysis patients were routinely dialyzed four to five hours three times weekly (blood flow, 200 to 300 mL/min; dialysate flow, 500 mL/min) using standard (N = 87) or high-flux (N = 61) HD membranes. For both HD and PD, the dialysate calcium concentration was 1.75 mmol/L. A lower dialysate calcium was used in patients with low parathyroid hormone (PTH) levels and low turnover osteopathy based on histologic diagnosis (N = 16). Calcium carbonate or calcium acetate were used as the primary phosphate binders. Moderate doses of aluminum hydroxide were added to the regimen only if calcium carbonate or calcium acetate resulted in intolerance or did not adequately control serum phosphorus (>5.5 mg/dL). Aluminum concentration in blood was monitored every year and was lower than 10 μg/L.

Active vitamin D metabolites (calcitriol/alphacalcidol) were given to all patients who showed no critical cal-

cium × phosphate product or no suppressed PTH (CRF I°, 1/−; CRF II°, 4/1; CRF III°, 10/3; HD, 90/27; PD, 10/3; RTX, 8/4). All RTX patients received prednisolone, cyclosporine A (adjusted to whole-blood concentrations between 120 and 180 ng/mL), and azathioprine (between 50 and 150 mg/day, adjusted to blood cell count). One RTX patient had been switched from cyclosporine to tacrolimus. The other medications included antihypertensive drugs, erythropoetin, iron, and insulin, as needed.

Assays

In all subjects, blood samples were collected under standardized nonfasting conditions and prior to dialysis (HD group). Samples were immediately centrifuged for 10 minutes at 4000 × *g* and then stored at −20°C until the assays were performed. Serum creatinine, calcium, and phosphate were measured by standard biochemical methods. To assess bone metabolism, we determined by radioimmunoassay (RIA): bone-specific alkaline phosphatase (B-ALP; Hybritech, Köln, Germany), osteocalcin (OSC; CIS, Dreieich, Germany), type-I collagen cross-linked carboxyterminal telopeptide (ICTP; Pharmacia, Freiburg, Germany), and carboxyterminal propeptide of type-I procollagen (PICP; Pharmacia, Freiburg, Germany). $25OHD_3$ and $1,25\text{-}(OH)_2D_3$ were measured by specific RIAs (Incstar, Stillwater, MN, USA) with a less than 2.5% cross-reactivity to other vitamin D metabolites. Intact PTH was measured within one week using a chemiluminescence immunometric assay (Nichols, CA, USA) with intra-assay and interassay coefficients of variation of less than 7%.

The components of the IGF system were measured according to published protocols [17, 22–26]. The normal range of IGF-I and IGFBP levels was obtained from 87 age- and sex-matched healthy blood donors [mean age, 57 ± 3 (18 to 70) years; female/male, 43/44) who were randomly recruited from the University Hospital Ulm.

Insulin-like growth factor-I was measured by an IGFBP-blocked specific RIA (IGF-R20; Mediagnost, Tuebingen, Germany) with a cross-reactivity to IGF-II of less than 0.05% and intra- and interassay coefficients of variation of less than 4 and 8%.

Free IGF-I levels were determined by a specific RIA (DSL, Frankfurt, Germany) with intra-assay and interassay coefficients of variation of less than 11%. There was no cross-reaction with IGF-II, insulin, proinsulin, and growth hormone.

Insulin-like growth factor-II was measured by a specific RIA (DSL) with intra-assay and interassay coefficients of variation of less than 7.5 and 10.5%. There was no cross-reaction with IGF-I, insulin, proinsulin, and IGFBP-2, -3, -4, and -5.

IGFBP-1 was measured by enzyme immunoassay (ELISA; Mediagnost) with intra-assay and interassay coefficients of variation of less than 4 and 8% and no cross-reaction with other IGFBPs.

IGFBP-2 was measured by a specific RIA with intra-assay and interassay coefficients of variation of less than 9 and 8% and no cross-reaction with IGFBP-1, -3, -4, -5, and -6 (DSL).

IGFBP-3 was determined by a specific RIA with intra-assay and interassay coefficients of variation of less than 3.5 and 7.5% and no cross-reaction with other IGFBPs (Mediagnost).

IGFBP-4 and IGFBP-5 were measured by specific RIAs as previously described [17, 26] with intra-assay and interassay coefficients of variation of less than 10% and no cross-reaction with other IGFBPs.

IGFBP-6 was determined by a specific RIA with intra-assay and interassay coefficients of variation of less than 11 and 10% and no cross-reaction with IGFBP-1, -2, -3, -4, and -5 (DSL).

Western immunoblot analysis

A 0.05 mL sample was diluted with 0.05 mL nonreducing sodium dodecyl sulfate (SDS)-dissociation buffer (0.125 mol/L Tris HCl, pH 6.8, 4% SDS, 20% glycerol) and then loaded onto a 1.5 mm discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and electrophoresed at 10 mA overnight through a 4% stacking gel and a 10 to 20% gradient separating gel. After SDS-PAGE, the proteins were transferred to 0.45 μm BA-S nitrocellulose membrane (Schleicher and Schuell, Keene, NH, USA), as previously described [26]. Nitrocellulose membranes were blocked for one hour with 5% nonfat dry milk, incubated for one hour with IGFBP-4 antiserum (1:2000 dilution), and then incubated for one hour with horseradish peroxidase-conjugated rabbit anti-guinea pig IgG (1:1000 dilution; Zymed Laboratories, San Francisco, CA, USA). Antigen-antibody reactions were visualized using ECL chemiluminescence reagents, as recommended by the manufacturer (Amersham Life Sciences, Arlington Heights, IL, USA).

Statistical analysis

Statistics were computed using SPSS. Results are expressed as mean ± SEM. Data were analyzed performing a one-way analysis of variance (ANOVA), followed by the Newman-Keuls test. The analysis was separated for the individual subgroups. Correlations between variables (Pearson's correlation coefficient) were assessed using univariate linear regression analysis. Analysis of covariance was performed to investigate the influence of age, sex, creatinine, PTH, and CRF stages on IGFBP-4 and IGFBP-5 levels. $P < 0.05$ was accepted as statistically significant.

Table 2. Serum levels of IGF-I, free IGF-I, IGF-II and IGFBP-1 to -6 in patients with different stages of chronic renal failure (CRF), end-stage renal failure (ESRF) treated by hemodialysis (HD), peritoneal dialysis (PD) or renal transplantation (RTX) compared to age-matched healthy control subjects (Controls) and patients with primary hyperparathyroidism (pHPT)

	Controls	pHPT	CRF I°	CRF II°	CRF III°	ESRF/HD	ESRF/PD	ESRF/RTX
N (Σ = 431)	87	25	29	48	37	148	27	30
Age *years*	57 ± 3	60 ± 3	56 ± 2.6	56 ± 2.6	57 ± 2.5	56 ± 3	45 ± 3	47 ± 2
IGF-I	135 ± 15 [45–288]	*n.d.*	99 ± 23 [69–129][a]	149 ± 25 [63–216]	123 ± 27 [48–231]	171 ± 66 [44–412]	*n.d.*	*n.d.*
Free IGF-I	2.7 ± 1.3 [0.94–9.4]	4.1 ± 0.7 [0.2–13.7][a]	4.2 ± 0.8 [0.3–17][a]	2.9 ± 0.3 [0.2–9.1]	3.6 ± 0.5 [0.4–11.7]	2.9 ± 0.3 [0.2–17.8]	2.2 ± 0.6 [0.2–15.7]	4.7 ± 0.8 [0.2–19.7][ad]
IGF-II	461 ± 37 [324–582]	*n.d.*	519 ± 46 [408–720]	517 ± 76 [354–774]	501 ± 48 [306–660]	925 ± 228 [432–1546][a]	*n.d.*	*n.d.*
IGFBP-1	2.3 ± 2.1 [0.1–17]	8.1 ± 2.2 [0.2–66][a]	15.6 ± 5 [1–104][ab]	25 ± 4 [2–91][ab]	26 ± 4 [3–80][ab]	33.2 ± 3 [0.9–172][ab]	25.7 ± 6 [1–98][ab]	17.9 ± 3 [1.4–60][ab]
IGFBP-2	474 ± 21 [121–1172]	907 ± 91 [290–1667][a]	1428 ± 185 [452–2771][a]	1847 ± 108 [873–3287][ab]	2491 ± 171 [908–4101][ab]	2914 ± 97 [984–6615][ab]	3561 ± 207 [793–5216][ab]	1428 ± 123 [428–2831][abd]
IGFBP-3	3087 ± 230 [1140–4968]	3853 ± 192 [2347–6519][a]	4610 ± 431 [1100–11734][a]	5120 ± 406 [1747–17817][a]	5267 ± 457 [1607–11355][a]	5269 ± 221 [1083–15758][a]	9721 ± 1695 [2673–45120][abc]	5349 ± 553 [1257–14533][ad]
IGFBP-4	396 ± 30 [160–796]	430 ± 34 [185–872]	1218 ± 227 [263–6364][ab]	1937 ± 207 [693–7559][ab]	2343 ± 245 [966–8878][ab]	4557 ± 410 [764–40749][abc]	7607 ± 1546 [1165–36692][abc]	1442 ± 146 [433–3670][abd]
IGFBP-5	454 ± 44 [319–713]	298 ± 21 [113–493][a]	313 ± 17 [149–500][a]	284 ± 19 [131–763][a]	320 ± 22 [151–773][a]	362 ± 61 [84–662]	315 ± 24 [109–544][a]	319 ± 21 [124–570][a]
IGFBP-6	99 ± 5 [41–198]	367 ± 72 [67–1313][a]	891 ± 131 [201–2041][ab]	986 ± 83 [185–2917][ab]	1097 ± 54 [645–1596][ab]	1666 ± 79 [316–6345][abc]	2497 ± 142 [1006–4240][abc]	755 ± 72 [273–1474][abd]

Data are mean values [range] ± SEM. *n.d.* is not determined.
[a] $P < 0.05$ vs. controls
[b] $P < 0.05$ vs. pHPT
[c] $P < 0.05$ vs. CRF III°
[d] $P < 0.05$ vs. PD

RESULTS

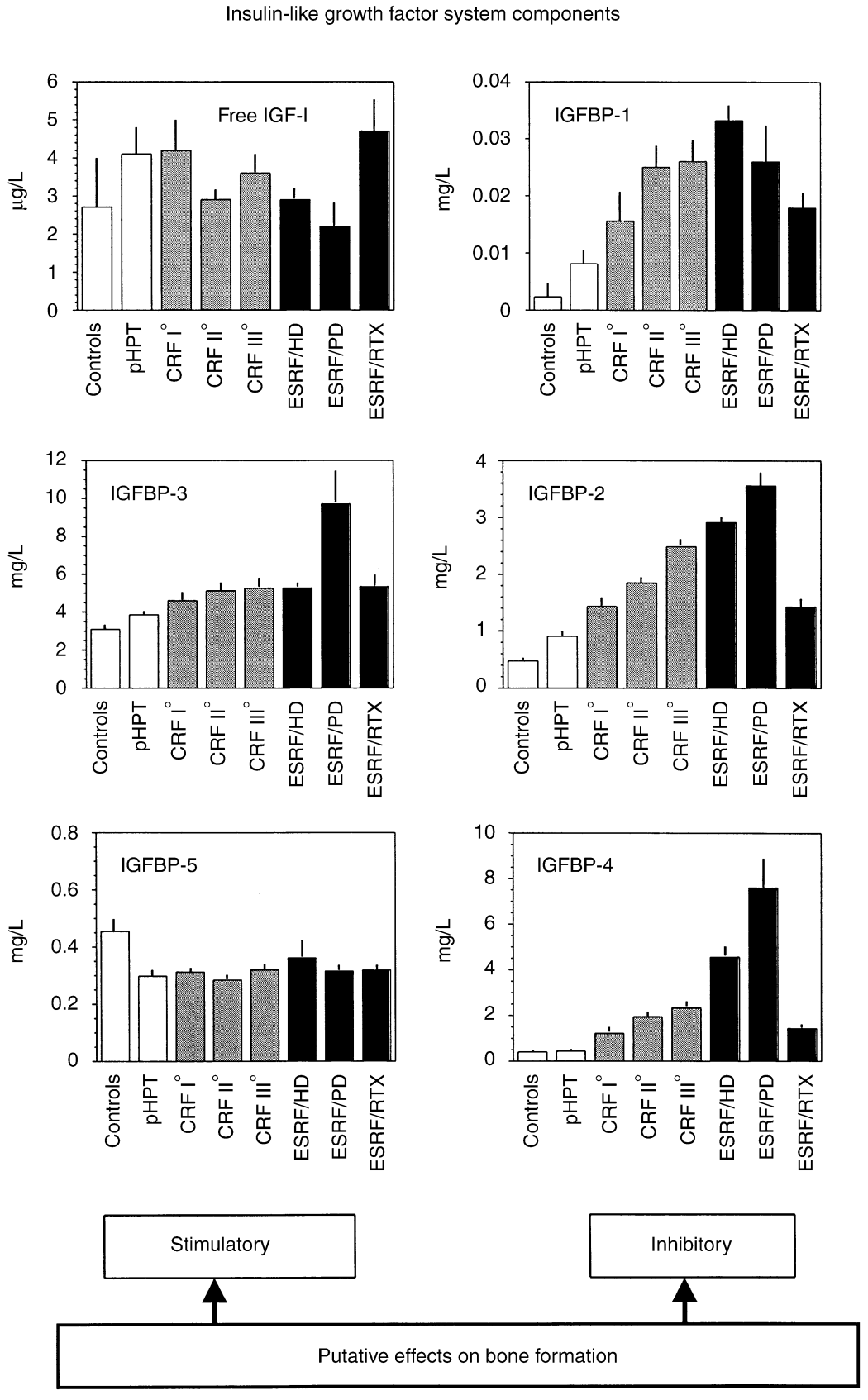
Insulin-like growth factor system components in the different patient groups

Table 2 summarizes data on circulating IGF system components in CRF patients compared with pHPT patients and age-matched healthy control subjects. As shown in Figure 1, with declining renal function, there was a significant increase in circulating IGFBP-1 (range 7- to 14-fold), IGFBP-2 (3- to 8-fold), IGFBP-3 (1.5- to 3-fold), IGFBP-4 (3- to 19-fold), and IGFBP-6 (8- to 25-fold). Generally, the disturbances in circulating IGF system components were most expressed in PD patients, whereas in RTX patients, circulating IGF system components tended to normalize. In pHPT patients, the elevation of IGFBP-1 (3.5-fold), IGFBP-2 (2-fold), IGFBP-3 (1.2-fold), and IGFBP-6 (4-fold) was significantly lower ($P < 0.01$) than in renal failure patients. HD patients revealed twofold higher IGF-II levels than the other groups ($P < 0.05$). Compared with control subjects, 1.5-fold higher levels of free IGF-I were found in pHPT, CRF I°, and RTX patients.

In contrast to other IGFBPs, IGFBP-5 levels were decreased (1.5-fold, $P < 0.05$) in all patient groups. In accordance with our previous findings [15], HD patients with diabetes mellitus ($N = 34$) showed lower IGFBP-5 levels (270 ± 16 ng/mL) than HD patients without diabetes mellitus (317 ± 10 ng/mL, $P < 0.05$). Interestingly, only renal failure patients showed increased IGFBP-4 levels. Immunoblot analysis revealed that the increase in immunoreactive IGFBP-4 represents an increase in both intact and fragmented forms of IGFBP-4. As shown in Figure 2, in PD patients (lane 1), approximately 50% of circulating IGFBP-4 could be attributed to lower molecular weight fragments, whereas patients with CRF I° (lane 3) showed no fragments.

To assess whether or not dialysis may influence IGFBP levels, blood samples were drawn before and after HD. In 20 HD patients, no differences could be observed for IGFBP-3 [before/after (ng/mL), 2682 ± 330/2686 ± 288], IGFBP-4 (2190 ± 36/2272 ± 53, respectively) and IGFBP-5 (253 ± 29/299 ± 42, respectively). Furthermore, there were also no significant differences in IGF system components between HD patients treated with high- or low-flux dialysis.

Univariate correlation analysis revealed that in all CRF patients, levels of free IGF-I correlated positively with IGFBP-3 ($r = 0.28$, $P < 0.05$) and IGFBP-5 ($r = 0.44$, $P < 0.005$) but negatively with IGFBP-1 ($r = -0.35$, $P < 0.01$), IGFBP-2 ($r = -0.31$, $P < 0.01$) and IGFBP-4 ($r = -0.28$, $P < 0.05$). Similar correlations were obtained with total IGF-I (data not shown). Figure 3 demonstrates the correlations between free IGF-I and IGFBP-4 (Fig. 3A) and IGFBP-5 (Fig. 3B) in the different groups of renal failure patients. Positive correlations were found between free IGF-I levels and total IGF-I ($r = 0.4$, $P < 0.01$) and between IGF-II and IGFBP-6 ($r = 0.4$, $P < 0.01$). Analysis of covariance showed that in CRF and ESRF patients, the different stages of renal insufficiency


Insulin-like growth factor system components
Free IGF-I
µg/L
IGFBP-1
mg/L
IGFBP-3
mg/L
IGFBP-2
mg/L
IGFBP-5
mg/L
IGFBP-4
mg/L
Controls
pHPT
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ESRF/PD
ESRF/RTX
Stimulatory
Inhibitory
Putative effects on bone formation

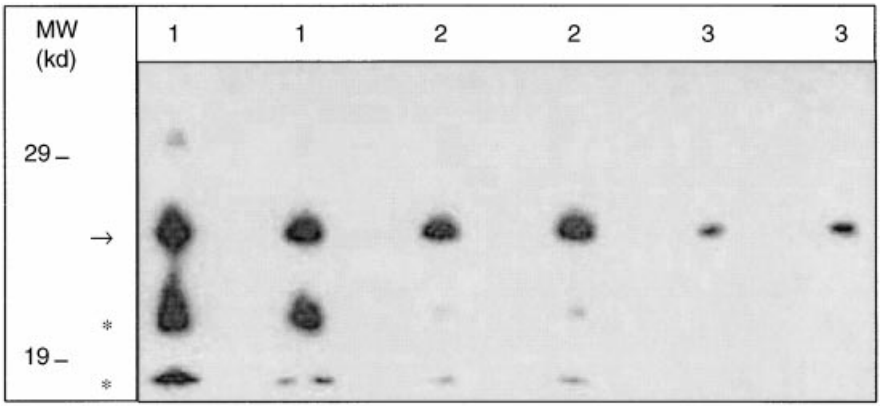

Fig. 2. Immunoblot analysis of insulin-like growth factor binding protein (IGFBP)-4 in sera of CRF patients with different IGFBP-4 levels. Sera from patients with high (1), medium (2), and low (3) IGFBP-4 values were separated by SDS-PAGE, transferred to nitrocellulose, and then immunoblotted with IGFBP-4 antiserum. The molecular weight (kd) of marker proteins is shown on the left. Intact IGFBP-4 (→) and IGFBP-4 fragments (star) are indicated. Mean IGFBP-4 concentrations (ng/mL; pooled sera of 5 patients in each group), determined by RIA, are 8400 (1), 1840 (2), and 620 (3).

correlate with the differences in IGFBP-4 levels (creatinine vs. IGFBP-4, $r = 0.58$, $P < 0.001$; Fig. 4), whereas gender was of minor influence (male patients showed 25% higher IGFBP-4 levels than females, $P < 0.05$). IGFBP-5 levels were influenced by age ($r = -0.23$, $P < 0.005$) but not by creatinine levels, the stage of renal insufficiency, gender, or PTH levels. Furthermore, IGFBP-2, -3, and -6 but not IGFBP-1 were significantly ($P < 0.05$) positive correlated with creatinine levels. Only IGFBP-6 showed a weak positive correlation with CRF duration ($r = 0.19$, $P < 0.05$).

Relationship between bone histology and IGF system components in hemodialysis patients

In 28 HD patients, bone histologic examination revealed mixed uremic bone disease (osteitis fibrosa plus osteomalacia) with ($N = 17$) or without ($N = 11$) osteopenia. In all cases, there were no signs of aluminum accumulation. The osteopenic subgroup showed reduced trabecular framework, thinning of trabecular elements, and disruption of trabecular continuity. Overall, osteopenic patients revealed significantly higher levels of IGFBP-2 and IGFBP-4 but lower levels of IGFBP-5, B-ALP, and OSC (Table 3). Significant correlations were observed between bone biopsy parameters, bone markers, and IGF system components (Table 4). As expected, there were positive correlations between endosteal fibrosis and osteoclastic surface on the one hand and serum levels of PTH, B-ALP, and OSC on the other hand. Osteoblastic surface correlated positively with serum levels of PTH, OSC, IGF-I, and IGFBP-5. A positive relationship was observed between osteoid thickness and PICP levels. Cortical bone thickness correlated positively with serum levels of OSC, IGF-II, IGFBP-3, and IGFBP-5 (Fig. 5A). Trabecular bone volume showed a positive relationship with IGFBP-5 levels (Fig. 5B) but was negatively correlated with IGFBP-2. Trabecular interconnections correlated positively with serum levels of B-ALP, OSC, IGF-I, IGFBP-5 (Fig. 5C), and IGFBP-6. A further positive correlation was obtained between the osteoblastic surface and IGFBP-5 (Fig. 5D). In contrast, IGFBP-4 correlated negatively with trabecular thickness and interconnections (Fig. 5 E, F). Finally, the bone marrow hematopoietic cell volume correlated positively with IGF-I, IGF-II, and IGFBP-6 levels and negatively with IGFBP-1 levels.

Bone markers in CRF and pHPT patients

As shown in Table 1, with deteriorating renal function PTH levels significantly increased but 1,25-D_3 levels decreased (CRF I° vs. other CRF/ESRF stages, $P < 0.01$). In contrast to renal failure patients, pHPT patients showed elevated levels of both PTH and 1,25-D_3, which is consistent with the physiological effect of PTH to stimulate 1,25-D_3 synthesis. Interestingly, B-ALP levels were highest in CRF I° patients and lowest in PD patients, which is consistent with previous reports showing a higher incidence of low turnover osteopathy in PD pa-

Fig. 1. Serum levels of insulin-like growth factor (IGF) system components (means ± SEM) and putative effects of bone formation in patients with different stages of chronic renal failure (CRF) and end-stage renal failure (ESRF) as compared with healthy controls and patients with primary hyperparathyroidism (pHPT). Serum levels of free IGF-I and IGFBP-1 to -5 were measured by specific immunometric assays. The stages of chronic renal failure (CRF) were defined as follows: CRF I°, mild renal insufficiency with creatinine up to 250 μmol/L and hyperparathyroidism but no further clinical signs of chronic renal failure ($N = 29$); CRF II°, moderate renal insufficiency with serum creatinine levels between 250 and 500 μmol/L and additional signs of chronic renal failure such as incipient anemia or metabolic acidosis ($N = 48$); CRF III°, serum creatinine levels above 500 μmol/L plus typical clinical manifestations of the complex uremic syndrome (for example, metabolic acidosis, fluid overload, electrolyte disturbances, accumulation of end-products of protein metabolism, $N = 37$); ESRF/HD = ESRF patients who were on maintenance renal replacement therapy by hemodialysis ($N = 148$).

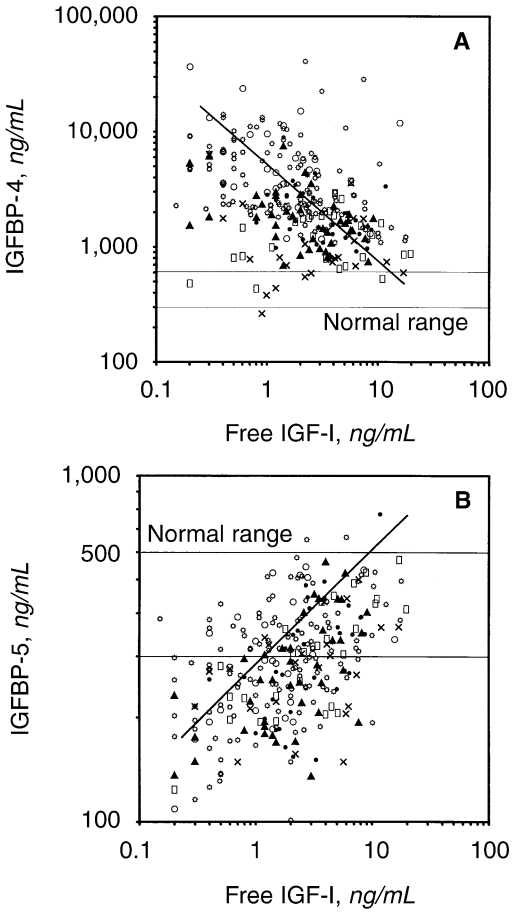

Fig. 3. Relationship between free IGF-I levels and IGFBP-4 (*A*) or IGFBP-5 (*B*) in patients with different stages of renal insufficiency [(×) CRF I°, (▲) CRF II°, (●) CRF III°, (✳) ESRF/HD, (○) ESRF/PD, (□) RTX]. The stages of chronic renal failure (CRF) were defined as described in the **Methods** section and in the legend to Figure 1. Free IGF-I and IGFBPs were measured by specific RIA. Normal ranges are indicated. Free IGF-I levels correlated negatively ($r = -0.28$, $P < 0.05$) with IGFBP-4 (A) and positively ($r = 0.44$, $P < 0.005$) with IGFBP-5 (B).

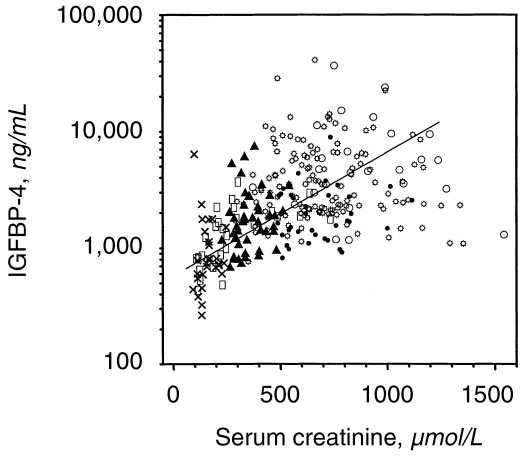

Fig. 4. Relationship between IGFBP-4 levels and serum creatinine in patients with different stages of renal insufficiency 1 [(×) CRF I°, (▲) CRF II°, (●) CRF III°, (✳) ESRF/HD, (○) ESRF/PD, (□) RTX]. The stages of chronic renal failure (CRF) were defined as described in the **Methods** section and in the legend to Figure 1. IGFBP-4 was measured by specific RIA and showed a positive correlation with serum creatinine ($r = 0.58$, $P < 0.001$).

tients. In contrast to B-ALP, levels of OSC and PICP markedly increased with renal insufficiency. This may be due to the reduced renal clearance of both markers and degraded fragments cross-reacting in the assays. Nevertheless, OSC and B-ALP showed significant positive correlations with PTH levels in all groups ($r \geq 0.45$, $P < 0.005$). The finding that PICP, a marker of bone matrix formation, was not increased in pHPT or CRF I° patients may indicate a negative uncoupling of bone formation to resorption in these groups.

IGF system components in dialysis patients with low or high bone turnover

Based on recent reports [27–32] that in dialysis patients, PTH levels within the normal range together with normal B-ALP levels are sensitive and specific (60 to 95%) in the diagnosis of LTO, whereas PTH levels > 200 pg/mL together with elevated B-ALP levels indicate high bone turnover (HTO), dialysis patients were divided into corresponding subgroups. Twenty-four dialysis patients matched the criteria of LTO, and 151 matched those of HTO. In 5 LTO patients and in 20 HTO patients, the diagnosis was confirmed by bone biopsy. As shown in Table 5, both groups showed similar clinical data. LTO patients showed significantly lower levels of PTH, B-ALP, OSC, and IGFBP-5 than HTO patients.

DISCUSSION

Our study provides the first analysis, to our knowledge, of immunoreactive serum levels of free and total IGF-I, IGF-II, and all six IGFBPs in a larger group of adult subjects with different forms of hyperparathyroidism and renal osteodystrophy. We demonstrated that with declining renal function, serum levels of all IGFBPs except IGFBP-5 considerably increase, whereas in most patients, IGF-I/-II serum levels were either not or only moderately elevated. Taking into account the increased half-life of IGFs in renal failure, normal serum levels of IGFs may indicate a reduced production [25, 33–35]. In children with CRF, a decreased hepatic IGF synthesis and markedly increased IGFBP levels have already been demonstrated [33, 36, 37]. In advanced CRF, other circumstances such as protein energy malnutrition and metabolic acidosis have already been demonstrated to reduce local and circulating IGF-I levels [38, 39].

The discrepancy between IGFs and IGFBPs is unique to CRF, as in all other clinical situations studied there is an approximate 1:1 ratio of total IGF (IGF-1 plus IGF-2) to IGFBPs. Previous studies demonstrated that

Table 3. Clinical data and IGF system components of HD patients with mixed uremic bone disease with or without histological signs of osteopenia

HD patients with mixed uremic bone disease	With osteopenia $N = 17$	Without osteopenia $N = 11$
Clinical data		
Age *years*	52 ± 3 [28–74]	48 ± 5 [33–71]
Duration of CRF *years*	8.5 ± 1.5 [1–28]	9.6 ± 2.4 [0.5–25]
Duration of dialysis *years*	3.8 ± 0.6 [0.5–8]	3.1 ± 0.5 [0.8–5]
Creatinine *58–110 μmol/L*	620 ± 60 [446–1120]	707 ± 84 [370–1153]
Bone markers		
PTH *11–54 pg/mL*	275 ± 61 [14–630]	340 ± 113 [2–1290]
B-ALP *7–17 ng/mL*	11 ± 2.1 [1–32]	27 ± 6.5 [8–81][b]
OSC *11–48 ng/mL*	31 ± 4.6 [9–83]	48 ± 8 [8–75][a]
PICP *40–200 ng/mL*	195 ± 20 [83–406]	232 ± 20 [92–357]
25-D_3 *10–60 ng/mL*	16 ± 5 [3–60]	22 ± 4 [16–49]
IGF system components		
Free IGF-I *0.94–9.4 ng/mL*	2.14 ± 0.3 [0.2–2.5]	3.03 ± 0.3 [0.3–4.4]
IGF-I *45–288 ng/mL*	163 ± 18 [44–286]	198 ± 33 [53–412]
IGF-II *324–582 ng/mL*	907 ± 43 [580–1144]	937 ± 70 [422–1125]
IGFBP-1 *0.1–17 ng/mL*	28 ± 3.8 [11.8–64]	35 ± 10 [4–112]
IGFBP-2 *121–1172 ng/mL*	2484 ± 134 [1527–3616]	1946 ± 188 [1111–2970][a]
IGFBP-3 *1140–4968 ng/mL*	4543 ± 695 [643–10779]	4862 ± 509 [1841–8428]
IGFBP-4 *160–796 ng/mL*	9288 ± 2238 [3619–21365]	6261 ± 1052 [1574–12616][a]
IGFBP-5 *319–713 ng/mL*	268 ± 17 [137–344]	320 ± 33 [185–509][a]
IGFBP-6 *41–198 ng/mL*	2673 ± 214 [794–3685]	2787 ± 411 [643–5229]

Data are mean values [range] ± SEM; reference values are in parentheses.
[a] $P < 0.05$, [b] $P < 0.005$

Table 4. Correlations between bone histologic parameters and laboratory findings

	CBTh	TBV	TTh	TIn	OTh	EF	ObS	OcS	BMHCV
Bone markers									
PTH	0.16	−0.11	0.02	0.23	0.26	0.38[a]	0.30[a]	0.56[b]	0.10
B-ALP	0.20	0.26	0.30[a]	0.40[a]	0.20	0.32[a]	0.23	0.20	−0.11
OSC	0.42[a]	0.23	0.18	0.49[b]	0.25	0.34[a]	0.33[a]	0.30[a]	0.16
PICP	0.22	0.13	0.12	0.14	0.47[b]	−0.01	0.27	−0.13	0.02
IGF system components									
Free IGF-I	0.03	−0.12	0.03	−0.09	−0.04	0.04	0.14	−0.24	0.18
IGF-I	0.14	0.15	0.17	0.44[a]	0.11	−0.01	0.30[a]	−0.14	0.41[a]
IGF-II	0.35[a]	−0.11	0.11	0.08	0.08	0.06	0.18	0.07	0.34[a]
IGFBP-1	−0.15	0.29	0.06	−0.22	−0.10	−0.09	−0.18	0.12	−0.51[b]
IGFBP-2	−0.26	−0.33[a]	−0.27	−0.41[a]	−0.23	0.20	−0.09	0.27	−0.17
IGFBP-3	0.37[a]	0.12	−0.22	0.05	−0.02	−0.15	0.18	−0.01	−0.10
IGFBP-4	−0.15	−0.22	−0.52[b]	−0.33[a]	−0.22	−0.12	0.23	−0.43[a]	0.05
IGFBP-5	0.35[a]	0.35[a]	0.17	0.38[a]	0.14	0.16	0.34[a]	−0.15	0.20
IGFBP-6	0.12	0.07	−0.10	0.39[a]	−0.02	0.08	0.11	0.07	0.58[b]

In 28 HD patients anterior iliac crest bone biopsies were performed using an electric drill. Transcortical bone samples of 2 to 3 cm in length and 5 mm in diameter were obtained. From Goldner stained sections the following parameters were assessed and graded according to a 4-point semiquantitative scale (−2 = markedly decreased, −1 = decreased, 0 = normal, 1 = increased): cortical bone thickness (CBTh), trabecular bone volume (TBV), trabecular thickness (TTh), trabecular interconnections (TIn), osteoid thickness (OTh), endosteal fibrosis (EF), osteoblastic surface (ObS), osteoclastic surface (OcS), and bone marrow hematopoietic cell volume (BMHCV). Coefficients of correlations (r) are given.
[a] $P < 0.05$
[b] $P < 0.005$

in CRF, circulating IGFBP-1 and -2 are truly increased, and those studies found no evidence that significant amounts of these binding proteins are degraded by specific proteases, whereas IGFBP-3 levels are overestimated by RIA because of proteolytic fragments (low molecular weight IGFBP-3 forms) with normal amounts of intact IGFBP-3 [25, 33, 35–37, 40]. In our study, the disturbances in the IGF system were more evident in PD than HD patients, whereas after RTX, IGFBP levels tended to normalize. Serum levels of IGFBP-4 and IGFBP-6 showed the most dramatic increase in ESRF patients. As demonstrated for IGFBP-4, immunoblot analysis revealed an increase in both intact and fragmented forms. The increased serum levels of IGFBP-4 may represent an increase in IGFBP-4 production or alterations in IGFBP-4 proteolysis [7, 41, 42]. Analysis of covariance revealed that IGFBP-4 levels are predominantly influenced by the stage of renal insufficiency. However, the decrease in renal clearance may not totally account for the increased IGFBP-4 levels because serum

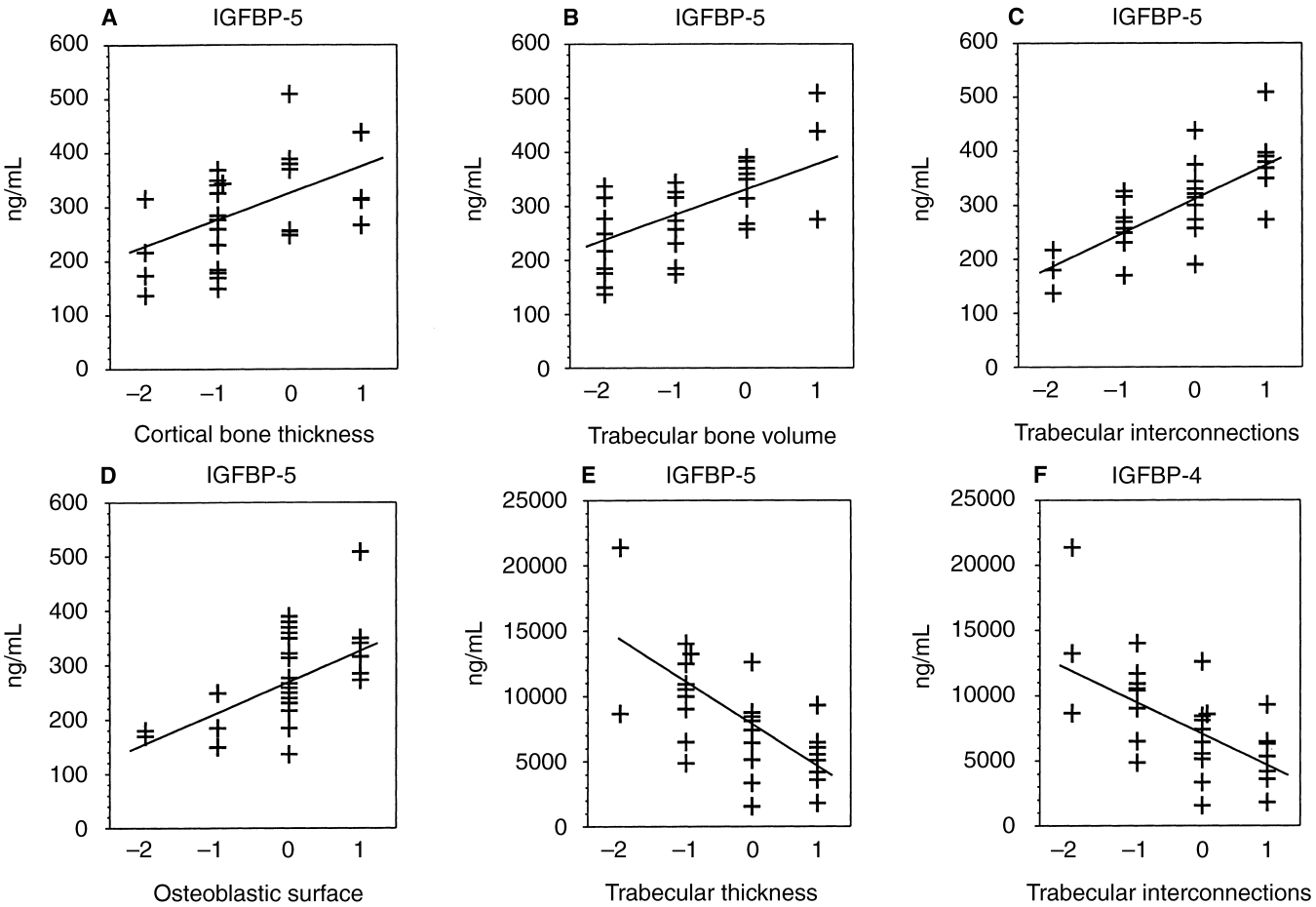

Fig. 5. Relationship between bone histologic findings and IGFBP-4/-5 levels in 28 hemodialysis patients. IGFBP-5 levels correlated positively with cortical bone thickness (*A*), trabecular bone volume (*B*), trabecular interconnections (*C*), and osteoblastic surface (*D*). In contrast, IGFBP-4 was negatively associated with trabecular thickness (*E*) and interconnections (*F*). Transcortical bone biopsies were performed at the iliac crest and histologic parameters were classified as follows: markedly decreased (−2), decreased (−1), normal (0), and increased (1). IGFBPs were measured by specific RIAs.

levels of IGFBP-5, which is of similar molecular weight, were not increased in CRF patients. Thus, the mechanisms that contribute to increased or diminished serum levels of low molecular weight IGFBPs in renal failure remain currently unknown. Of particular interest to this discussion is that the increase in the relative levels of free low molecular weight IGFBPs in the serum of CRF patients could inhibit IGF actions in the local tissues because these IGFBPs can easily cross the vascular endothelium [8]. This may contribute to end-organ resistance to growth hormone and IGF-I in uremia [33, 43]. The hypothesis that at least some of the circulating IGF inhibitors are of small molecular weight is supported by the finding that HD treatment temporarily increases bioactivity of IGF-I [36]. Because we did not measure significant differences in circulating IGFBP-3 (44 kd), IGFBP-4 (25 kd), and IGFBP-5 (29 kd) before and after HD, as well as in patients treated with high- or low-flux dialysis, the postulated IGF inhibitors should have a lower molecular weight than intact IGFBPs (for example, 18 kd fragment of IGFBP-4; Fig. 2) [5–7, 18].

Our study is the first report, to our knowledge, on serum levels of IGFBP-4 and -5 in a large number of adult patients with different stages of CRF. Both IGFBPs are considered to be important regulators of bone cell function. *In vitro* studies have shown that IGFBP-4 is a potent inhibitor of IGF actions on bone cells by preventing IGFs from binding to their receptors [9], whereas IGFBP-5 may exert anabolic effects on osteoblasts by an IGF-independent mechanism involving IGFBP-5–specific binding sites [44]. Although the exact functional role of serum IGFBP-4 and IGFBP-5 is not clear at this time, increased IGFBP-4 serum levels have been reported in hip fracture patients with elevated serum PTH levels [16]. Interestingly, our study demonstrated lower IGFBP-5 but higher IGFBP-4 levels in renal osteodystrophy patients with histologic signs of osteopenia and lower IGFBP-5 levels in LTO patients. It might

Table 5. Clinical data, bone markers, and IGF system components in hemodialysis patients with low (LTO) or high (HTO) bone turnover

HD patients	LTO $N = 24$	HTO $N = 151$
Clinical data		
Age *years*	57 ± 3 [39–81]	54 ± 2 [28–81]
Duration of dialysis *years*	4.1 ± 1 [1–18]	4.6 ± 0.4 [1–21]
Diabetes mellitus *% of patients*	21	19
PD/HD *% of patients*	20/80	15/85
Creatinine *58–110 μmol/L*	681 ± 30 [441–1072]	731 ± 25 [360–1541]
Bone markers		
PTH *11–54 pg/mL*	27 ± 3 [2–54]	390 ± 29 [62–1470][b]
B-ALP *7–17 ng/mL*	11 ± 3 [2–17]	25 ± 1 [4–353][a]
OSC *11–48 ng/mL*	31 ± 5 [10–94]	205 ± 35 [18–2874][a]
PICP *40–200 ng/mL*	179 ± 19 [76–323]	247 ± 12 [83–1066]
IGF system components		
Free IGF-I *0.94–9.4 ng/mL*	2.17 ± 0.7 [0.3–15.7]	2.68 ± 0.2 [0.3–17.8]
IGFBP-1 *0.1–17 ng/mL*	47 ± 11 [3–219]	34 ± 3 [1–157]
IGFBP-2 *121–1172 ng/mL*	2743 ± 269 [1438–5216]	2602 ± 81 [1125–6615]
IGFBP-3 *1140–4968 ng/mL*	4860 ± 458 [1561–11394]	6227 ± 385 [1083–45120]
IGFBP-4 *160–796 ng/mL*	5852 ± 1513 [1807–36692]	4957 ± 425 [764–40749]
IGFBP-5 *319–713 ng/mL*	264 ± 20 [84–494]	315 ± 9 [101–662][a]
IGFBP-6 *41–198 ng/mL*	2102 ± 144 [1210–3702]	2228 ± 82 [316–6059]

Data are mean values [range] ± SEM; reference values are in brackets.
[a] $P < 0.05$
[b] $P < 0.005$

be hypothesized that not only the locally produced IGFBP-4 and IGFBP-5 [4, 9, 44], but also their endocrine levels may have significant biological effects on bone [15–18]. This view is supported by significant correlations between bone histologic findings and serum levels of bone markers and IGF system components. In this regard, our findings demonstrate that in general, serum levels of stimulatory IGF system components show positive correlations with histologic parameters of bone formation, whereas the serum levels of inhibitory IGFBPs (IGFBP-2 and IGFBP-4) show positive correlations with histologic indices of bone loss. Furthermore, we observed that the bone marrow hematopoietic cell volume correlated positively with IGF-I, IGF-II, and IGFBP-6 levels but negatively with IGFBP-1. In the same patients, blood hemoglobin values showed significant positive correlations with levels of free IGF-I ($r = 0.30$, $P < 0.05$) and IGF-II ($r = 0.39$, $P < 0.05$). Taken together, these findings indicate that an imbalance between IGFs and IGFBPs (that is, decreased IGF bioavailability) may not only contribute to bone loss but also to anemia in renal failure patients.

As a functional consequence, the imbalance in circulating IGF system components in uremia together with a complex resistance to both growth hormone and IGF-I significantly contributes to growth failure in children and experimental animals [34, 43, 45]. Our study provides evidence for an association between bone histology, bone metabolic markers, and circulating levels of IGF system components in renal osteodystrophy patients. Previous studies support this hypothesis showing that IGF-I administration stimulates bone formation parameters in animal and human models [46, 47], whereas disruption of IGF-I results in severe impairment of bone formation in mice and humans [48]. In our study, HD patients with osteopenia showed lower levels of free and total IGF-I than patients without osteopenia according to our and other previous findings in patients with osteopenia and osteoporosis [12, 13, 15]. Other investigators reported no correlations between circulating IGFs and bone formation parameters in a smaller number of predialysis [49] or dialysis patients [50]. A recent study in 533 premenopausal and postmenopausal women in whom bone biopsies from the iliac crest were obtained demonstrated that bone matrix IGF-I was positively associated with histomorphometric and biochemical parameters of bone formation and with cancellous bone volume [51]. These authors further reported that women with a bone matrix IGF-I concentration two standard deviations above the mean had a 20% higher bone volume than women with a bone matrix IGF-I concentration two standard deviations below the mean and that serum IGF-I was weakly associated with bone matrix IGF-I when adjusted for the serum concentration of IGF binding protein-3. In this study, we found that serum levels of stimulatory IGFBP-5 showed significant positive correlations with cortical bone thickness, trabecular bone volume, trabecular interconnections, and osteoblastic surface (Fig. 5). This finding with IGFBP-5 is significant for several reasons: (*a*) IGFBP-5, a growth hormone dependent IGFBP, has been shown to stimulate bone formation parameters both *in vitro* and *in vivo* [52]; (*b*) IGFBP-5 actions in bone may involve both IGF-dependent and IGF-independent mechanisms [7]; and (*c*)

among the six high affinity IGFBPs, IGFBP-5 is the only IGFBP whose levels in serum were not influenced by the stage of renal insufficiency (Table 2).

It is well known that PTH can cause bone formation [53] as well as bone loss [54]. The anabolic effects of PTH [53, 55] are at least partially mediated by an increase in osteoblastic IGF-I synthesis, which inhibits apoptosis of osteoblast precursor cells. It is important to note that the anabolic action of PTH involves its pulsatile secretion pattern, which is disturbed in both advanced HPT and CRF. An interesting finding of our study was that pHPT and CRF I° patients showed similar elevations of both PTH and free IGF-I, whereas in advanced renal failure, IGF synthesis may not keep up with rising PTH. Consistent with previous reports [28–32, 56], dialysis patients with low turnover osteopathy showed significantly lower levels of PTH and B-ALP than patients with HTO. Our study revealed for the first time, to our knowledge, that patients with low turnover osteopathy also had significantly lower levels of the stimulatory IGFBP-5. Patients with long-term pHPT and sHPT develop significant bone loss but regain bone after parathyroidectomy [54, 57]. Elevated PTH levels may contribute to bone loss through an increase in inhibitory IGF system components such as IGFBP-4 [16]. Finally, patients with severe sHPT show a broad spectrum of skeletal and extraskeletal symptoms [18, 54, 57, 58]. In these patients, it seems appropriate to call PTH a "uremic toxin" [59]. In terms of clinical practice, more specific serum markers of bone formation and resorption are required in addition to levels of intact PTH, B-ALP, and OSC [56]. In our study, IGFBP-5 correlated positively with all histologic signs of bone formation and therefore might gain importance as a more specific marker of bone formation. The following examples in HD patients (age-matched, no diabetes mellitus) in whom bone markers (Table 1 and Table 2 show reference values of bone markers) and bone histology were performed may support this hypothesis: (*a*) in two older patients (A/B; age, 61/62 years; dialysis duration, 6/8 years) showing only slight differences in B-ALP (25/23 ng/mL) and OSC (58/44 ng/mL) but different levels of PTH (289/622 pg/mL), PICP (357/177 ng/mL), and IGFBP-5 (314/137 ng/mL), bone biopsy revealed normal bone formation in patient A but significant bone loss in patient B. (*b*) In two young patients (C/D; age, 17/29 years; dialysis duration, 8/3 years) revealing B-ALP (8/7 ng/mL) and OSC (24/17 ng/mL) in the low normal range and normal or slightly elevated PTH (14/79 pg/mL) and PICP (193/295 ng/mL), histologic signs of osteopenia were found in patient C showing also a low IGFBP-5 (277 ng/mL) but not in patient D (IGFBP-5, 509 ng/mL). Larger prospective studies are necessary to assess the value of IGFBP-5 in predicting the histologic abnormalities in bone.

In conclusion, our data indicate a functional relationship between circulating IGF system components and bone metabolism in patients with different forms of hyperparathyroidism and renal osteodystrophy. The elevated free IGF-I levels in early renal failure may compensate for the inhibitory IGFBP action, whereas in ESRF, the predominant increase of inhibitory rather than stimulatory IGFBPs (Fig. 1) may diminish IGF bioactivity. The major findings of this study support the idea that especially the markedly elevated IGFBP-4 levels, which are characteristic for CRF but not pHPT, and the lower IGFBP-5 levels may contribute to a diminished bone formation in renal osteodystrophy. The characterization of circulating growth factors and cytokines may not only elucidate the complex pathophysiology of renal osteodystrophy [60], but also provide tools for the differential diagnosis and therapy.

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APPENDIX

Abbreviations used in this article are: B-ALP, bone alkaline phosphatase; CRF, chronic renal failure; CRF I°, mild renal insufficiency; CRF II°, moderate renal insufficiency; CRF III°, complex uremic syndrome; ESRF, end-stage renal failure; HD, hemodialysis; HTO, high bone turnover; IDDM, insulin-dependent diabetes mellitus; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; LTO, low bone turnover; OSC, osteocalcin; pHPT, primary hyperparathyroidism; PD, peritoneal dialysis; PICP, procollagen type 1 C-peptide; PTH, parathyroid hormone; RIA, radioimmunoassay; RTX, renal transplantation; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; sHPT, secondary hyperparathyroidism.

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