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Effect of chronic renal failure on bone turnover and bone alkaline phosphatase isoforms

PER MAGNUSSON,[1] CHRISTOPHER A. SHARP, MARTIN MAGNUSSON, JUHA RISTELI, MICHAEL W.J. DAVIE, and LASSE LARSSON

Bone and Mineral Metabolic Unit, Division of Clinical Chemistry, Department of Biomedicine and Surgery, Linköping University Hospital, Linköping, Sweden; Charles Salt Centre, Robert Jones and Agnes Hunt Orthopaedic and District Hospital NHS Trust, Oswestry, Shropshire, England, United Kingdom; Division of Nephrology, Department of Medicine and Care, Linköping University Hospital, Linköping, Sweden; and Department of Clinical Chemistry, University of Oulu, Oulu, Finland

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Background. Biochemical markers of bone turnover are used to monitor metabolic bone disease associated with renal failure. We have applied a comprehensive panel of markers to patients with chronic renal failure (CRF), with particular focus on the isoforms of bone alkaline phosphatase (BALP).

Methods. Twenty CRF patients undergoing hemodialysis ($N = 9$) and peritoneal dialysis ($N = 11$) were measured for serum parathyroid hormone (PTH), osteocalcin, total ALP, and four BALP isoforms (B/I, B1x, B1, and B2) by high-performance liquid chromatography. These BALP isoforms were also compared with BALP measured by three commercial immunoassays (Alkphase-B, Tandem-R Ostase, and Tandem-MP Ostase). Type I collagen turnover was assessed by serum samples using the type I procollagen intact amino- and carboxy-terminal propeptides (PINP and PICP) and two fragments (ICTP and CrossLaps) derived from the carboxy-terminal telopeptide of mature matrix collagen by different degradative pathways.

Results. Mean levels of bone turnover markers were elevated in CRF, with marked increases in those markers, osteocalcin, ICTP, and CrossLaps, cleared by the kidney. Total ALP activities were increased corresponding to elevated B/I and B2 isoform levels. The B1 isoform level was not significantly different from healthy controls. B1x was detected in 60% of the patients but was not resolved in healthy individuals. Kendall's tau rank correlation showed that B1x correlated significantly ($P < 0.05$) with B1 (0.53) and PINP (0.55), and was the only marker to correlate with PTH (0.49). B1x was not significantly correlated with any of the commercial BALP immunoassays. Interestingly, the immunoassay calibrators contained high activities of the B/I peak (39 to 80%) compared with human serum (4%).

Conclusion. There are selective differences between the BALP isoforms in CRF compared with healthy adults. The commercial BALP immunoassays are comparable with each other but are unable to distinguish the BALP isoform-specific differences in CRF patients.

Renal bone disease, common in chronic renal failure (CRF), is a multifactoral disorder of bone remodeling encompassing a spectrum of histologically classified disorders ranging from high-turnover osteitis fibrosa through adynamic osteopathies to low-turnover bone disease [1]. Accurate classification of the type of renal bone disease is achieved through bone histomorphometry after iliac crest biopsy. However, less invasive approaches, including the use of biochemical markers of bone turnover and various skeletal imaging methods such as dual-energy x-ray absorptiometry and ^{18}F-fluoride positron emission tomography, are under investigation [2–4].

Markers of bone turnover such as bone alkaline phosphatase (BALP) and osteocalcin are considered specific for osteoblast-related processes [5]. Serum total ALP is frequently used to monitor bone metabolism in patients with renal failure, as ALP is one of the few bone markers that is not influenced by variations in renal function. Approximately 95% of the total ALP activity is derived from bone and liver sources, which occur in a ratio of approximately 1:1 in serum from healthy adults [6]. Both BALP and liver ALP (LALP) are encoded by the tissue nonspecific gene locus and are referred to as isoforms of the same isoenzyme. BALP functions as an ectoenzyme attached to the osteoblast cell membrane by a hydrophobic glycosylphosphatidylinositol (GPI) anchor [7, 8]. BALP has been reported to be necessary for the initiation of mineralization but not for the continuation of the process [9–11]; however, the precise function of BALP has still to be elucidated.

[1] Present address: Musculoskeletal Disease Center (151), Jerry L. Pettis Memorial VA Medical Center, 11201 Benton Street, Loma Linda, CA 92357, USA.

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In this study, we have used a previously described weak anion-exchange high-performance liquid chromatography (HPLC) method that can resolve at least six different ALP isoforms in serum from healthy adults: three BALP (B/I, B1, and B2) and three LALP (L1, L2, and L3) isoforms [12, 13]. An additional BALP isoform (B1x) has recently been observed in human bone tissue samples and was also found in serum from patients with CRF [14]. A higher B1x activity was found in trabecular bone in comparison with cortical bone, which is in line with the other BALP isoforms. By several different identification procedures, B1x was verified to be a soluble (anchorless) ALP isoform of bone origin circulating in CRF patients. A possible kidney ALP origin, or intestinal ALP type from human kidney, was also ruled out. B1x has not been observed in healthy individuals or in any other group of patients. However, the presence of B1x in healthy subjects (and in patients) cannot be excluded since its intermediate retention, and possible lower activity than either of the B/I and B1 peaks, may confound its detection [14].

In healthy adults, the three BALP isoforms, B/I, B1, and B2, account on average for 4, 16, and 37%, respectively, of the total serum ALP activity [14]. In serum, the minor fraction B/I is not a pure BALP isoform, as it coelutes with intestinal ALP and is composed, on average, of bone (70%) and intestinal (30%) ALP. Both of the two major BALP isoforms found in serum, B1 and B2, are soluble, as they have no remaining fragments of the in situ GPI cell membrane anchor, and the differences are likely due to different glycosylation patterns [14]. We have recently reported that cortical bone has approximately twofold higher activity of B1 compared with B2, and conversely, the B2 activity is approximately twofold higher in trabecular bone compared with B1 [14]. Selective differences between these BALP isoforms have previously been described in several disease states including the following: growth hormone deficiency [6], hypophosphatasia, hypophosphatemic vitamin D-resistant rickets, stress fractures [12, 15], metastatic bone disease [16], Paget's disease [abstract; Sharp et al, *J Bone Miner Res* 14(Suppl 1):S540, 1999], and during the pubertal growth spurt [17].

We also investigated serum osteocalcin and several markers of collagen turnover. De novo collagen formation was assessed by measuring both the intact amino- and carboxy-terminal propeptides of type I procollagen (PINP and PICP). Extracellular cleavage of these allows the newly synthesized collagen to be incorporated into the fibrillar matrix. Both PICP and intact PINP may have added utility in renal failure since their clearance from the circulation is independent of renal function [18]. Type I collagen breakdown was assessed by measuring two serum-based markers of collagen degradation: type I collagen carboxy-terminal telopeptide (ICTP) and CrossLaps. Both structures are derived from the carboxy-terminal telopeptide region of trivalently cross-linked type I collagen, although the epitope and the origin of the fragments measured are different. The CrossLaps assay detects cathepsin K generated degradation products, whereas the ICTP antigenicity is destroyed by cathepsin K [19]. Serum ICTP is most likely produced by the action of matrix metalloproteinases.

This study was primarily designed to investigate the BALP isoforms, in particular the B1x isoform, in patients with CRF undergoing dialysis therapy and to compare these isoforms with commercially available immunoassays of BALP. We also assessed some of the most recently developed assays of type I collagen turnover and their relationships with the BALP isoforms.

METHODS

Subjects

The study group with CRF was composed of 20 adult patients, 9 males and 11 females, with a mean age of 63 years (range 33 to 87 years). All were on chronic dialysis therapy: hemodialysis ($N = 9$; 5 males and 4 females), with a median (range) disease duration of 9 (4 to 26) years and treatment duration of 23 (4 to 126) months, or peritoneal dialysis ($N = 11$; 4 males and 7 females), with a disease duration of 13 (1 to 27) years and a treatment duration of 13 (5 to 89) months. Underlying nephropathy types were 10 with chronic glomerulonephritis, 4 with diabetic nephropathy, 2 with nephrosclerosis, 1 with chronic tubulointerstitial nephropathy, and 3 of unknown origin. All patients were treated with calcium carbonate (phosphate binder) and alphacalcidol (vitamin D analogue). None were treated with corticosteroids, and no patients received aluminum-containing phosphate binders. For both peritoneal and hemodialysis, the dialysate calcium concentration was 1.75 mmol/L. All blood samples were drawn predialysis, and no significant differences were found between those patients on peritoneal and hemodialysis therapy with respect to age, disease duration, or dialysis duration.

The control group for serum BALP isoforms was composed of 153 healthy adults, 45 males and 108 females, with a mean age of 46 years (range 21 to 90 years), and the control group for serum osteocalcin was composed of 1121 healthy adults, 190 males and 931 females, with a mean age of 53 years (range 25 to 90 years). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of Linköping University Hospital (Linköping, Sweden).

Biochemical determinations

The routine serum analytes, creatinine, urea, phosphate, and calcium corrected for serum albumin were determined using a Hitachi 917 analyzer (Boehringer

Mannheim GmbH, Mannheim, Germany). Serum intact parathyroid hormone (PTH) was determined by a two-site immunoradiometric assay (IRMA; Nichols Institute, San Juan Capistrano, CA, USA), with a reported reference interval for healthy adults of 12 to 65 ng/L [20]. Serum osteocalcin was determined by an in-house competitive radioimmunoassay (RIA) procedure using a polyclonal antiserum raised in a rabbit against purified bovine osteocalcin [21].

Serum PICP, PINP, and ICTP were determined by RIA (Orion Diagnostica, Oulunsalo, Finland). Reported reference intervals for serum PICP and PINP in healthy adults were 38 to 202 μg/L and 20 to 76 μg/L for men and 50 to 170 μg/L and 19 to 84 μg/L for women, respectively [22, 23]. Similarly, reference intervals for serum ICTP in healthy adults were 1.8 to 5.0 μg/L for men and women [24]. We also used the recently developed serum CrossLaps™ One Step enzyme-linked immunosorbent assay (ELISA; Osteometer Biotech A/S, Herlev, Denmark), which is reported to measure degradation products of mature cross-linked type I collagen [25]. This assay detects a different antigenic epitope to the ICTP assay and relies on the presence of two cross-linked carboxy-terminal telopeptide α1 chains, each bearing an octapeptide sequence containing a β-isomerized aspartate residue. The reported serum CrossLaps levels (mean ± SD) in a healthy adult female reference population were 1748 ± 740 pmol/L in premenopausal and 2952 ± 1325 pmol/L in postmenopausal women [25].

Serum total ALP activity was measured on a Hitachi 917 analyzer (Boehringer Mannheim GmbH) [26]. The BALP isoforms B/I, B1x, B1, and B2 were determined by a previously described HPLC method [12, 13]. In summary, the BALP isoforms were separated using a gradient of 0.6 mol/L sodium acetate at pH 7.6 on a weak anion-exchange column, SynChropak AP300 (250 × 4.6 mm I.D.; Eichrom Technologies, Inc./SynChrom, Inc., Darien, IL, USA), optimized for BALP isoform analysis [6]. The effluent was mixed online with the substrate solution (1.8 mmol/L p-nitrophenyl phosphate in a 0.25 mol/L diethanolamine buffer, pH 10.1), and the ensuing reaction took place in a packed bed postcolumn reactor at 37°C. The formed product (p-nitrophenol) was then directed online through the detector set at 405 nm. The areas under each peak were integrated, and the total ALP activity was used to calculate the relative activity of each of the detected BALP isoforms.

We also measured total serum BALP using three different commercially available assays: Alkphase-B® from Metra Biosystems, Inc. (Mountain View, CA, USA), Tandem®-R Ostase™, and Tandem®-MP Ostase™, both from Hybritech, Inc./Beckman Coulter (San Diego, CA, USA). The monoclonal antibodies against BALP used in these immunoassays were all prepared by immunizing mice with BALP purified from the SaOS-2 osteosarcoma cell line, and all assays are standardized against BALP purified from SaOS-2 cells [27, 28]. Alkphase-B is a two-stage assay based on a single monoclonal antibody, coated to microtiter strips, which captures BALP. In the second stage, the activity of the captured BALP is determined with a colorimetric endpoint. Results are reported in activity units (U/L). Details of the procedure and assay performance characteristics are described elsewhere [28–30]. Reported serum BALP reference intervals with the Alkphase-B assay for healthy adults were 15.0 to 41.3 U/L for men and 11.6 to 30.6 U/L for women [28]. Tandem-R Ostase is a solid-phase, two-site IRMA using two monoclonal antibodies directed against different epitopes of BALP. Results are reported in mass units (μg/L). Details of the procedure and assay performance characteristics are described elsewhere [31, 32]. Reported serum BALP reference intervals with the Tandem-R Ostase assay for healthy adults were 5 to 22 μg/L for men and women [32]. We also used Tandem-MP Ostase, a recently reported single monoclonal antibody microplate-based enzyme immunoassay [33]. The BALP monoclonal antibody used in this assay is reported to be identical to that used in the Tandem-R Ostase IRMA [27, 33]. Reported serum BALP reference intervals with the Tandem-MP Ostase assay for healthy adults are the same as for the Tandem-R Ostase assay (discussed previously in this article) [32, 33].

Statistical analysis

All calculations were performed with the StatView 5.0 program (SAS Institute Inc., Cary, NC, USA). Nonparametric statistics were used because the distributions were not Gaussian according to the Kolmogorov-Smirnov test. The Mann–Whitney test was used to test for differences between the groups of healthy individuals and patients with CRF and to test for sex-related differences within the groups. The Kruskal–Wallis test was used if more than two groups were compared. To measure the association of linear relationship, we calculated Kendall's tau rank correlation coefficient. A difference was considered statistically significant at $P < 0.05$ for all statistical tests.

RESULTS

Routine serum analytes, PTH, osteocalcin, and markers of type I collagen turnover

The serum levels of the routine analytes were (mean ± SEM): creatinine, 668 ± 34 μmol/L; urea, 18.2 ± 0.8 mmol/L; phosphate, 1.86 ± 0.13 mmol/L; and calcium corrected for serum albumin concentration, 2.57 ± 0.05 mmol/L. The mean serum PTH level was increased (mean ± SEM) to 304 ± 78 ng/L in the CRF group. The serum osteocalcin reference interval by mean ± 2 SD for healthy adults ($N = 1121$), with a mean age of 53 years (range 25 to 90 years), was <30 μg/L. No significant

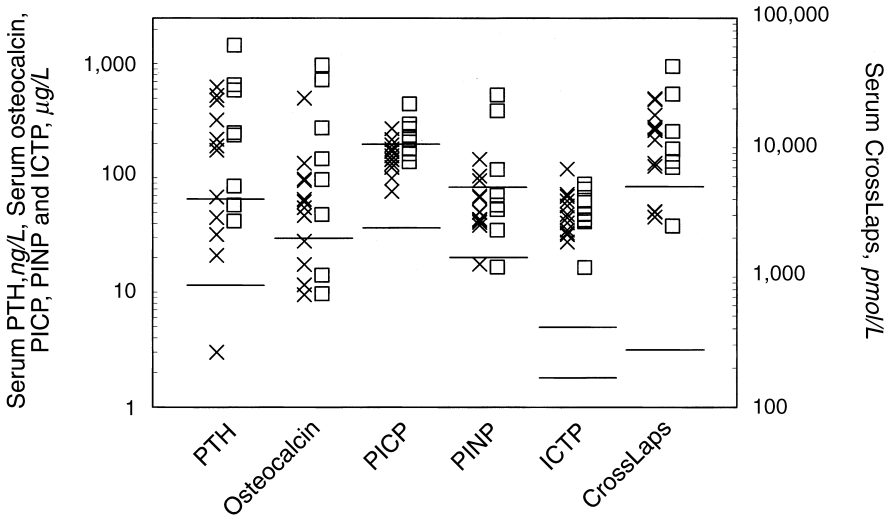

Fig. 1. Scatter diagram showing the parathyroid hormone (PTH), osteocalcin, markers of type I collagen formation (PICP and PINP), and type I collagen degradation (ICTP and CrossLaps) concentrations for the patient group with CRF. Symbols are: (×) chronic renal failure (CRF) patients with B1x (N = 12); (□) CRF patients with no detectable B1x (N = 8). The horizontal lines indicate the upper and lower reference limits. Note that only the upper reference limit is shown for osteocalcin.

differences were found between those undergoing hemodialysis and peritoneal dialysis therapy or between females and males for any of the measured routine analytes and markers of bone turnover. Moreover, no significant Kendall's tau rank correlation was observed with disease duration or dialysis duration. Of the bone turnover markers, those cleared by renal filtration (osteocalcin, ICTP, and CrossLaps) had the highest serum levels in comparison with the reference intervals of healthy adults. In particular, values for ICTP in all 20 patients were markedly elevated with serum concentrations on average tenfold greater than the upper reference limit (Fig. 1).

BALP by commercial immunoassay kits

Bone alkaline phosphatase values in the CRF group were (mean ± SEM) 17.3 ± 2.9 U/L for the Alkphase-B assay, 23.1 ± 4.8 µg/L for the Tandem-R Ostase assay, and 21.6 ± 4.0 µg/L for the Tandem-MP Ostase assay (Fig. 2). The Kruskal–Wallis test showed no significant difference between the three assays. However, 17 of the 20 BALP values were in the lower quartile or below the reported reference interval for the Alkphase-B assay, but not for either of the two other immunoassays (Fig. 2).

Using HPLC, we analyzed the BALP isoform profiles of the high-level calibrators from the Alkphase-B and Tandem-R Ostase immunoassay kits (Fig. 3). Both of these calibrators were found to have high activities of the B/I fraction (Alkphase-B 80% and Tandem-R Ostase 39%) in comparison with human serum, where the B/I component has the lowest activity of all of the ALP isoforms (on average 4% of the total ALP activity). Hence, it is not surprising that the B/I peak has the highest degree of linear relationship among the BALP isoforms with these commercial immunoassays (Table 1).

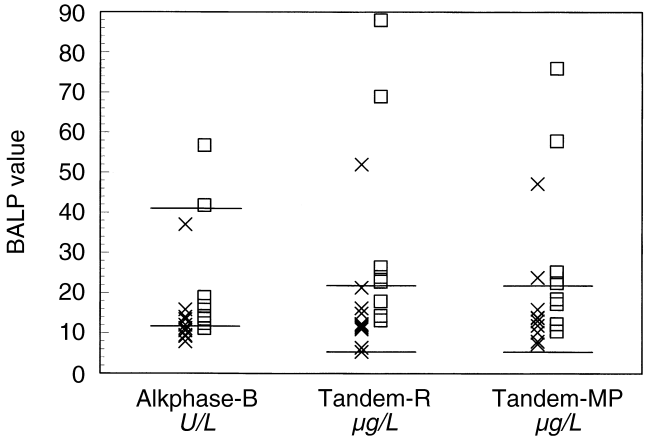

Fig. 2. Scatter diagram showing the BALP values for the immunoassays Alkphase-B, Tandem-R Ostase, and Tandem-MP Ostase for the patient group with CRF. Symbols are: (×) CRF patients with B1x (N = 12); (□) CRF patients with no detectable B1x (N = 8). The horizontal lines indicate the upper and lower reference limits.

BALP isoforms by HPLC

Six ALP isoforms were separated and quantitated by HPLC in each of the healthy adult serum samples investigated: three BALP (B/I, B1, and B2) and three LALP (L1, L2, and L3) isoforms. Total ALP and ALP isoform reference intervals by 2.5 and 97.5% for healthy adults (N = 153), with a mean age of 46 years (range 21 to 90 years), were total ALP, 1.6 to 4.1 µkat/L; B/I, 0.04 to 0.17 µkat/L; B1, 0.20 to 0.62 µkat/L; B2, 0.34 to 1.69 µkat/L; B1/B2 ratio, 0.23 to 0.72; L1, 0.12 to 0.74 µkat/L; L2, 0.36 to 0.83 µkat/L; and L3, 0.10 to 0.34 µkat/L. No significant differences were found between the different age decades (Kruskal–Wallis test). The median value of the B/I isoform was higher in females than in males ($P <$ 0.001, 0.09 and 0.07 µkat/L, respectively). No other sex-related differences were found.

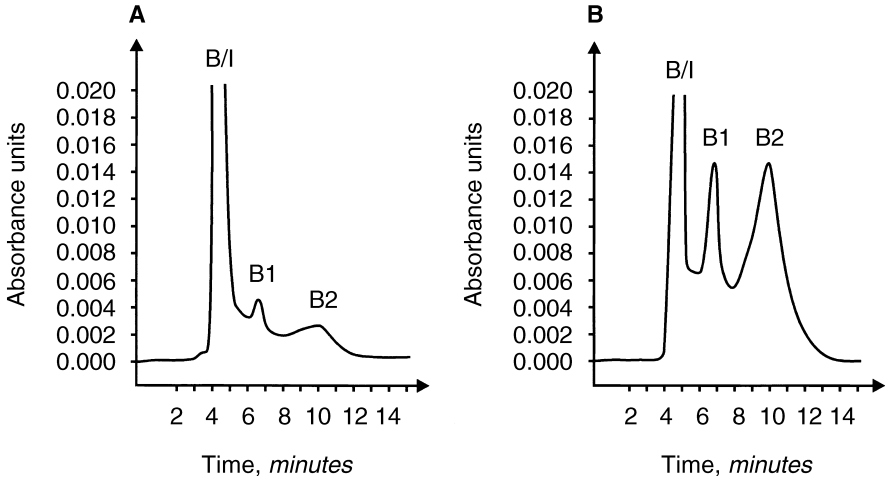

Fig. 3. Chromatographic BALP profiles. (*A*) High BALP calibrator of the Alkphase-B assay (140 U/L). Peaks, activities, retention times, and peak heights, in order of elution are as follows: B/I, 112 U/L, 4.83 min, 0.0662 absorbance units; B1, 13 U/L, 6.77 min, 0.0040 absorbance units; B2, 15 U/L, 9.92 min, 0.0022 absorbance units. (*B*) High BALP calibrator of the Tandem-R Ostase assay (120 μg/L). Peaks, activities, retention times, and peak heights in order of elution are as follows: B/I, 47 μg/L, 4.87 min, 0.0505 absorbance units; B1, 24 μg/L, 6.83 min, 0.0148 absorbance units; B2, 49 μg/L, 9.82 min, 0.0149 absorbance units.

Table 1. Correlation coefficients between PTH and biochemical markers of bone turnover in patients with CRF

		Total	BALP isoforms by HPLC					BALP by immunoassays			Type I collagen markers			
	PTH	ALP	B/I	B1x[a]	B1	B2	B1/B2	Alkphase-B	Tandem-R	Tandem-MP	PICP	PINP	ICTP	CrossLaps
Total ALP	0.07													
B/I	0.25	0.51[c]												
B1x	0.49[b]	0.70[c]	0.47[b]											
B1	0.22	0.64[e]	0.57[d]	0.53[b]										
B2	−0.10	0.66[e]	0.23	0.32	0.37[b]									
B1/B2	0.23	−0.30	0.06	0.09	0.05	−0.57[d]								
Alkphase-B	0.24	0.51[c]	0.48[c]	0.41	0.44[c]	0.38[b]	−0.11							
Tandem-R	0.17	0.36[b]	0.55[d]	0.12	0.41[b]	0.30	−0.19	0.67[e]						
Tandem-MP	0.20	0.57[d]	0.55[d]	0.42	0.48[c]	0.36[b]	−0.12	0.81[e]	0.63[e]					
PICP	0.08	0.22	0.37[b]	0.09	0.24	0.24	−0.26	0.23	0.39[b]	0.20				
PINP	0.20	0.39[b]	0.32[b]	0.55[b]	0.27	0.34[b]	−0.25	0.52[c]	0.44[c]	0.46[c]	0.34[b]			
ICTP	0.07	0.40[b]	0.07	0.42	0.18	0.54[d]	−0.49[c]	0.19	0.03	0.22	0.30	0.37[b]		
CrossLaps	0.12	0.25	0.20	0.36	0.06	0.26	−0.31	0.39[b]	0.18	0.45[c]	0.23	0.68[e]	0.42[b]	
Osteocalcin	0.23	0.23	0.33[b]	0.33	0.17	0.17	−0.12	0.51[c]	0.43[c]	0.45[c]	0.35[b]	0.70[e]	0.20	0.56[d]

Abbreviations are in the **Appendix**. Patients with CRF, $N = 20$.
[a] Patients with CRF expressing B1x, $N = 12$
[b] $P < 0.05$, [c] $P < 0.01$, [d] $P < 0.001$, and [e] $P < 0.0001$, by Kendall's tau rank correlation

The total ALP activity (mean ± SEM, 4.5 ± 0.51 μkat/L) was significantly ($P < 0.0001$) increased in CRF patients in comparison with healthy adults, and this increase was due to elevated levels of B/I (0.17 ± 0.04 μkat/L, $P < 0.05$) and B2 (1.73 ± 0.32 μkat/L, $P < 0.01$; Fig. 4). Moreover, no significant difference was observed for the B1 isoform (0.42 ± 0.05 μkat/L, $P = 0.54$) between the CRF patients and healthy adults. No significant differences were found for any of the BALP isoforms between the groups of hemodialysis and peritoneal dialysis or between females and males within the CRF group. Only the B1/B2 ratio showed a significant Kendall's tau rank correlation with disease duration (0.40, $P < 0.02$), and no significant correlation was observed with the duration of dialysis treatment.

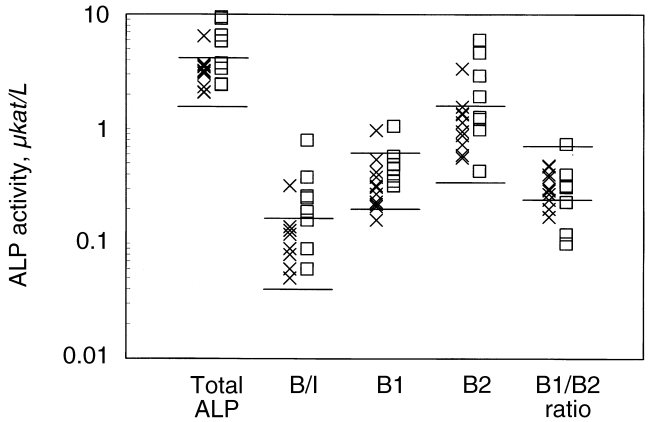

Fig. 4. Scatter diagram showing the total ALP and BALP isoform activities for the patient group with CRF. Symbols are: (×) CRF patients with B1x ($N = 12$); (□) CRF patients with no detectable B1x ($N = 8$). The horizontal lines indicate the upper and lower reference limits.

Detection of the BALP isoform B1x

The additional BALP isoform B1x was detected in serum from 12 (60%) of the CRF patients. The B1x

Table 2. Comparison between CRF patients displaying the B1x BALP isoform (B1x+) and patients with no detectable B1x (B1x−)

	B1x+	B1x−	*P* value[a]
Total ALP *μkat/L*	3.2 (2.1–6.5)	4.8 (2.4–10.0)	NS (0.08)
B/I *μkat/L*	0.08 (0.05–0.32)	0.22 (0.06–0.80)	<0.05
B1 *μkat/L*	0.32 (0.16–0.97)	0.42 (0.32–1.06)	<0.05
B2 *μkat/L*	1.24 (0.56–3.36)	1.60 (0.43–6.01)	NS (0.25)
B1/B2 ratio	0.28 (0.17–0.48)	0.27 (0.10–0.74)	NS (0.76)
Alkphase-B *U/L*	11.2 (7.9–37.0)	15.4 (11.2–56.8)	<0.05
Tandem-R *μg/L*	12.0 (5.4–52.0)	23.4 (13.2–88.0)	<0.01
Tandem-MP *μg/L*	13.4 (7.3–47.2)	20.5 (10.5–76.0)	NS (0.054)
PTH *ng/L*	183 (3.0–626)	244 (42–1450)	NS (0.25)
Osteocalcin *μg/L*	58 (9.5–503)	122 (9.7–976)	NS (0.25)
PICP *μg/L*	152 (76–272)	226 (139–446)	<0.05
PINP *μg/L*	50 (18–146)	64 (17–536)	NS (0.40)
ICTP *μg/L*	46 (28–120)	48 (17–88)	NS (0.62)
CrossLaps *pmol/L*	12550 (2909–24000)	9818 (2484–42500)	NS (0.93)

Values are given as median, with the minimum and maximum values in parentheses. NS is not significant. Other abbreviations are listed in the **Appendix**.
[a]Mann–Whitney test between CRF patients displaying the B1x BALP isoform (B1x+, $N = 12$) and CRF patients with no detectable B1x (B1x−, $N = 8$)

isoform has previously been detected in extracts of human bone but has not been resolved in serum from healthy individuals. The original observation of the B1x peak, including a chromatographic profile and characterization of this bone ALP peak, is reported elsewhere [14]. The B1x isoform did not show a significant correlation with any of the three immunoassays, and moreover, patients with detectable B1x were not those expressing the highest levels of BALP measured by the immunoassays (Fig. 2). Notably, B1x was the only biochemical marker of bone turnover that had a significant correlation with PTH (0.49, $P < 0.05$; Table 1).

Dividing the CRF patients into two groups based on those patients displaying the B1x BALP isoform (B1x+, $N = 12$) and patients with no detectable B1x (B1x−, $N = 8$) revealed several differences. Patients with B1x were older, with a median age of 71 years (range 51 to 87 years), versus 54 years (range 33 to 79 years) of age ($P < 0.05$), and had, in general, lower median levels of the markers of bone turnover (Table 2 and Figs. 1, 2, and 4). In particular, median levels of the B/I and B1 isoforms and BALP, measured using the Alkphase-B and Tandem-R Ostase assay kits, and PICP levels were significantly lower in those patients with B1x. However, no significant differences were found for creatinine, urea, phosphate, corrected calcium, PTH, dialysis duration, or disease duration.

DISCUSSION

We have investigated circulating markers of bone turnover, in particular the isoforms of BALP, in a group of CRF patients managed by either peritoneal dialysis or hemodialysis. To our knowledge, our study provides the first analysis and comparison of BALP isoforms measured by HPLC and commercial BALP immunoassays, in addition to other serum markers of type I collagen turnover used in the assessment of bone metabolism in CRF.

Previous studies have reported elevated markers of type I collagen turnover in CRF patients [3, 34, 35], although the utility of these makers has been doubted [36]. The usefulness of PICP in assessing bone formation has been considered in hemodialysis patients with renal bone disease by Hamdy et al, who reported significant correlations between histologically determined bone formation rates and the bone formation markers PICP, total ALP, and osteocalcin [34]. Furthermore, they deduced that formation markers within reference intervals for healthy individuals were associated with normal bone formation rates, which suggests that the majority of our CRF patients with formation marker levels within the reference limits could have a normal bone formation rate.

Circulating levels of osteocalcin, ICTP, and CrossLaps, which are cleared by the kidney, were elevated in the majority of our patients. The interpretation of serum osteocalcin in dialysis patients has been proved difficult, since many of the immunoassays employ polyclonal antibodies with broad specificities and the ability to detect several immunologic fragments, including the intact molecule [37, 38]. Thus, reduced renal clearance inevitably leads to elevated osteocalcin levels, although the effectiveness of the various dialysis techniques to remove osteocalcin, as well as ICTP and CrossLaps, is uncertain. Peritoneal dialysis has been shown to reduce serum osteocalcin in children with end-stage renal disease [39]. Similarly, serum levels of pyridinoline cross-links are reduced by hemodialysis, indicating clearance of these cross-links and, by inference, their cross-linked peptides from the circulation by this technique [40]. The clinical usefulness of osteocalcin and type I collagen telopeptides in assessing bone turnover in CRF is questionable, although relative osteocalcin levels have been considered to distinguish low from high-turnover bone disease [41–43].

Parathyroid hormone was analyzed using a commercial assay originally reported to detect the intact (1-84 PTH) circulating molecule. However, it has recently

been demonstrated that a fragment (most likely 7-84 PTH) interferes significantly with this assay [44, 45]. Thus, the PTH values reported in our study might be higher than the true circulating levels of intact 1-84 PTH because of the presence of this amino-terminal truncated fragment of inactive 7-84 PTH. In addition to the more obvious methodological aspects, this finding also will presumably have implications for the treatment of renal bone disease.

No significant differences were observed between the three different BALP immunoassays. As shown in the chromatographic profiles of the commercial calibrators (Fig. 3), these kits are calibrated to a large degree against the B/I fraction. In serum from healthy subjects, B/I has a lower activity than either the B1 and B2 isoforms and represents, on average, only 4% of the total ALP activity measured. Some differences have been reported between these BALP immunoassays in groups of patients that express different ratios of these BALP isoforms, which could be explained by differences between the antibodies used in these immunoassays [46–48]. A worldwide workshop facilitated by the International Society of Oncodevelopmental Biology and Medicine (ISOBM) has recently begun to evaluate the properties, specificity, and target epitopes of all available antibodies to BALP. The results of this workshop may provide useful information about the nature and location of the epitopes, including new information about the different BALP isoforms [abstract; Ärlestig et al, *Tumor Biol* 21(Suppl 1):87, 2000].

In addition to differences between the BALP isoforms patterns in clinical situations [6, 12, 15–17], we also identified bone compartment-specific differences. Cortical bone was found to have approximately twofold higher activity of B1 compared with B2, whereas trabecular bone had approximately twofold higher activity of B2 compared with B1 [14]. The detection of B1x in some patients with renal disease may be related to differences in the metabolism of different bone compartments or, more specifically, the effects of PTH on osteoblast populations within these compartments. Generalized thinning of cortical bone due to increased net endocortical resorption has recently been reported in a group of patients with histologically classified osteitis fibrosa and osteomalacia [49]. The influence of PTH and site-specific differences in bone turnover, and their possible effect on the osteoblast synthesis of BALP isoforms, requires further investigation, in addition to studying a larger cohort of renal patients classified by bone histomorphometry.

Most interestingly, we found that a number of the CRF patients expressed a recently described BALP isoform termed B1x, which was not detectable in the healthy control group and which has only been identified in extracts of human bone [14]. To date, the B1x has not been observed in healthy children or adults or in any of the bone-related pathologies investigated [6, 12, 15–17]. However, the presence of B1x in these sera cannot be excluded since its intermediate retention time, and possible lower activity than either of the B/I and B1 peaks, may confound its detection [14].

The BALP isoform B1x could be related to secondary hyperparathyroidism, which is common in dialysis patients, since B1x was the only marker of bone turnover that showed a significant association with PTH. Alternatively, B1x may indicate low-turnover adynamic bone disease as those patients expressing B1x had lower median values for 12 of the measured analytes (Table 2), which may reflect suppressed osteoblast activity. It is unlikely that B1x is related to the other type of low-turnover bone disease in renal bone disease, that is, osteomalacia, since this is usually accompanied by increased ALP levels [5]. In general, this was not the case for the CRF patients with B1x. Further investigations of patients with primary and secondary hyperparathyroidism and histologically defined bone disease are needed before these findings are adequately explained.

In summary, our data show that there are selective differences between the BALP isoforms in patients with CRF in comparison with healthy adults. The increased total ALP activity was largely due to increased activities of the BALP isoforms B/I and B2 in several patients. The BALP isoform B1x was detected in 60% of the patients, but was not detected in the control group of healthy individuals. B1x did not show a significant correlation with any of the three BALP immunoassays; however, it was the only biochemical marker of bone turnover that had a significant correlation with PTH. Interestingly, the immunoassay calibrators had relatively high activities of the B/I fraction (39 to 80%) in comparison with serum from healthy individuals (4%). The three commercially available BALP immunoassays are comparable with each other, but are unable to distinguish the BALP isoform specific differences in this set of patients with CRF. Future investigations should include immunoassay analysis of purified BALP isoform fractions in order to characterize the specificity of these antibodies to the different BALP isoforms. Furthermore, the BALP isoform B1x should be evaluated as a potential marker of low turnover adynamic bone disease accurately classified by bone histomorphometry.

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Reprint requests to Per Magnusson, Ph.D., Musculoskeletal Disease Center (151), Jerry L. Pettis Memorial VA Medical Center, 11201 Benton Street, Loma Linda, California 92357, USA.
E-mail: per.magnusson@med.va.gov

APPENDIX

Abbreviations used in this study are: ALP, alkaline phosphatase; BALP, bone alkaline phosphatase; CRF, chronic renal failure; ELISA, enzyme-linked immunosorbent assay; GPI, glycosylphosphatidylinositol; HPLC, high-performance liquid chromatography; ICTP, carboxy-terminal telopeptide of type I collagen; IRMA, immunoradiometric assay; LALP, liver alkaline phosphatase; PICP, carboxy-terminal propeptide of type I procollagen; PINP, intact amino-terminal propeptide of type I procollagen; PTH, parathyroid hormone; RIA, radioimmunoassay.

REFERENCES

1. Hruska KA, Teitelbaum SL: Renal osteodystrophy. *N Engl J Med* 333:166–174, 1995
2. Messa C, Goodman WG, Hoh CK, *et al:* Bone metabolic activity measured with positron emission tomography and [^{18}F] fluoride ion in renal osteodystrophy: Correlation with bone histomorphometry. *J Clin Endocrinol Metab* 77:949–955, 1993
3. Ureña P, de Vernejoul M-C: Circulating biochemical markers of bone remodeling in uremic patients. *Kidney Int* 55:2141–2156, 1999
4. Rix M, Andreassen H, Eskildsen P, *et al:* Bone mineral density and biochemical markers of bone turnover in patients with predialysis chronic renal failure. *Kidney Int* 56:1084–1093, 1999
5. Calvo MS, Eyre DR, Gundberg CM: Molecular basis and clinical application of biological markers of bone turnover. *Endocr Rev* 17:333–368, 1996
6. Magnusson P, Degerblad M, Sääf M, *et al:* Different responses of bone alkaline phosphatase isoforms during recombinant insulin-like growth factor-I (IGF-I) and during growth hormone therapy in adults with growth hormone deficiency. *J Bone Miner Res* 12:210–220, 1997
7. Fedde KN, Lane CC, Whyte MP: Alkaline phosphatase is an ectoenzyme that acts on micromolar concentrations of natural substrates at physiologic pH in human osteosarcoma cells. *Arch Biochem Biophys* 264:400–409, 1988
8. Hooper NM: Glycosyl-phosphatidylinositol anchored membrane enzymes. *Clin Chim Acta* 266:3–12, 1997
9. Tenenbaum HC: Levamisole and inorganic pyrophosphate inhibit β-glycerophosphate induced mineralization of bone formed in vitro. *Bone Miner* 3:13–26, 1987
10. Bellows CG, Aubin JE, Heersche JNM: Initiation and progression of mineralization of bone nodules formed in vitro: The role of alkaline phosphatase and organic phosphate. *Bone Miner* 14:27–40, 1991
11. Barling PM, Gupta DK, Lim CEL: Involvement of phosphodiesterase I in mineralization: Histochemical studies using antler from red deer (Cervus elaphus) as a model. *Calcif Tissue Int* 65:384–389, 1999
12. Magnusson P, Löfman O, Larsson L: Determination of alkaline phosphatase isoenzymes in serum by high-performance liquid chromatography with post-column reaction detection. *J Chromatogr* 576:79–86, 1992
13. Magnusson P, Löfman O, Larsson L: Methodological aspects on separation and reaction conditions of bone and liver alkaline phosphatase isoform analysis by high-performance liquid chromatography. *Anal Biochem* 211:156–163, 1993
14. Magnusson P, Larsson L, Magnusson M, *et al:* Isoforms of bone alkaline phosphatase: Characterization and origin in human trabecular and cortical bone. *J Bone Miner Res* 14:1926–1933, 1999
15. Magnusson P, Löfman O, Toss G, Larsson L: Determination of bone alkaline phosphatase isoforms in serum by a new high-performance liquid chromatography assay in patients with metabolic bone disease. *Acta Orthop Scand* 66(Suppl 266):203–204, 1995
16. Magnusson P, Larsson L, Englund G, *et al:* Differences of bone alkaline phosphatase isoforms in metastatic bone disease and discrepant effects of clodronate on different skeletal sites indicated by the location of pain. *Clin Chem* 44:1621–1628, 1998
17. Magnusson P, Häger A, Larsson L: Serum osteocalcin and bone and liver alkaline phosphatase isoforms in healthy children and adolescents. *Pediatr Res* 38:955–961, 1995
18. Risteli J, Risteli L: Products of bone collagen metabolism, in *Dynamics of Bone and Cartilage Metabolism: Principles and Clinical Applications*, edited by Seibel MJ, Robins SP, Bilezikian JP, London, Academic Press, 1999, pp 275–287
19. Sassi M-L, Eriksen H, Risteli L, *et al:* Immunochemical characterization of assay for carboxyterminal telopeptide of human type I collagen: Loss of antigenicity by treatment with cathepsin K. *Bone* 26:367–373, 2000
20. Nussbaum SR, Zahradnik RJ, Lavigne JR, *et al:* Highly sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. *Clin Chem* 33:1364–1367, 1987
21. Worsfold M, Sharp CA, Davie MWJ: Serum osteocalcin and other indices of bone formation: An 8-decade population study in healthy men and women. *Clin Chim Acta* 178:225–235, 1988
22. Melkko J, Niemi S, Risteli L, Risteli J: Radioimmunoassay of the carboxyterminal propeptide of human type I procollagen. *Clin Chem* 36:1328–1332, 1990
23. Melkko J, Kauppila S, Niemi S, *et al:* Immunoassay for intact amino-terminal propeptide of human type I procollagen. *Clin Chem* 42:947–954, 1996
24. Risteli J, Elomaa I, Niemi S, *et al:* Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: A new serum marker of bone collagen degradation. *Clin Chem* 39:635–640, 1993
25. Rosenquist C, Fledelius C, Christgau S, *et al:* Serum CrossLaps One Step ELISA: First application of monoclonal antibodies for measurement in serum of bone-related degradation products from C-terminal telopeptides of type I collagen. *Clin Chem* 44:2281–2289, 1998
26. Scandinavian Society for Clinical Chemistry and Clinical Physiology: Committee on enzymes: Recommended methods for the determination of four enzymes in blood. *Scand J Clin Lab Invest* 33:291–306, 1974
27. Hill CS, Wolfert RL: The preparation of monoclonal antibodies which react preferentially with human bone alkaline phosphatase and not liver alkaline phosphatase. *Clin Chim Acta* 186:315–320, 1990
28. Gomez B Jr, Ardakani S, Ju J, *et al:* Monoclonal antibody assay for measuring bone-specific alkaline phosphatase activity in serum. *Clin Chem* 41:1560–1566, 1995
29. Withold W, Schulte U, Reinauer H: Method for determination of bone alkaline phosphatase activity: Analytical performance and clinical usefulness in patients with metabolic and malignant bone diseases. *Clin Chem* 42:210–217, 1996
30. Milligan TP, Park HR, Noonan K, Price CP: Assessment of the performance of a capture immunoassay for the bone isoform of alkaline phosphatase in serum. *Clin Chim Acta* 263:165–175, 1997
31. Garnero P, Delmas PD: Assessment of the serum levels of bone alkaline phosphatase with a new immunoradiometric assay in patients with metabolic bone disease. *J Clin Endocrinol Metab* 77:1046–1053, 1993
32. Panigrahi K, Delmas PD, Singer F, *et al:* Characteristics of a two-site immunoradiometric assay for human skeletal alkaline phosphatase in serum. *Clin Chem* 40:822–828, 1994
33. Broyles DL, Nielsen RG, Bussett EM, *et al:* Analytical and clinical performance characteristics of Tandem-MP Ostase, a new immunoassay for serum bone alkaline phosphatase. *Clin Chem* 44:2139–2147, 1998
34. Hamdy NAT, Risteli J, Risteli L, *et al:* Serum type I procollagen peptide: A non-invasive index of bone formation in patients on haemodialysis? *Nephrol Dial Transplant* 9:511–516, 1994
35. Jensen PB, Rasmussen HB, Pedersen FB: Serum concentration of the aminopropeptide of type I procollagen in patients on haemo- and peritoneal dialysis. *APMIS* 105:371–377, 1997

36. Franke S, Lehmann G, Abendroth K, *et al:* PICP as bone formation and NTx as bone resorption marker in patients with chronic renal failure. *Eur J Med Res* 3:81–88, 1998
37. Garnero P, Grimaux M, Seguin P, Delmas PD: Characterization of immunoreactive forms of human osteocalcin generated in vivo and in vitro. *J Bone Miner Res* 9:255–264, 1994
38. Masters PW, Jones RG, Purves DA, *et al:* Commercial assays for serum osteocalcin give clinically discordant results. *Clin Chem* 40:358–363, 1994
39. Gundberg CM, Hanning RM, Liu YA, *et al:* Clearance of osteocalcin by peritoneal dialysis in children with end-stage renal disease. *Pediatr Res* 21:296–300, 1987
40. Ibrahim S, Mojiminiyi S, Barron JL: High-performance liquid chromatographic determination of pyridinium crosslinks in serum, urine and dialysate of patients in chronic renal failure. *Ann Clin Biochem* 33:31–35, 1996
41. Malluche HH, Faugere MC, Fanti P, Price PA: Plasma levels of bone Gla-protein reflect bone formation in patients on chronic maintenance dialysis. *Kidney Int* 26:869–874, 1984
42. Charhon SA, Delmas PD, Malaval L, *et al:* Serum bone Gla-protein in renal osteodystrophy: Comparison with bone histomorphometry. *J Clin Endocrinol Metab* 63:892–897, 1986
43. Ureña P, Ferreira A, Kung VT, *et al:* Serum pyridinoline as a specific marker of collagen breakdown and bone metabolism in hemodialysis patients. *J Bone Miner Res* 10:932–939, 1995
44. Lepage R, Roy L, Brossard J-H, *et al:* A non-(1-84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples. *Clin Chem* 44:805–809, 1998
45. Slatopolsky E, Finch J, Clay P, *et al:* A novel mechanism for skeletal resistance in uremia. *Kidney Int* 58:753–761, 2000
46. Woitge HW, Seibel MJ, Ziegler R: Comparison of total and bone-specific alkaline phosphatase in patients with nonskeletal disorders or metabolic bone diseases. *Clin Chem* 42:1796–1804, 1996
47. Price CP, Milligan TP, Darte C: Direct comparison of performance characteristics of two immunoassays for bone isoform of alkaline phosphatase in serum. *Clin Chem* 43:2052–2057, 1997
48. Magnusson P, Degerblad M, Sääf M, *et al:* Different isoforms of bone alkaline phosphatase exist. *J Bone Miner Res* 13:760–761, 1998
49. Schober H-C, Han Z-H, Foldes AJ, *et al:* Mineralized bone loss at different sites in dialysis patients: Implications for prevention. *J Am Soc Nephrol* 9:1225–1233, 1998