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Aluminum, Iron, Lead, Cadmium, Copper, Zinc, Chromium, Magnesium, Strontium, and Calcium Content in Bone of End-Stage Renal Failure Patients

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Background: **Little is known about trace metal alterations in the bones of dialysis patients or whether particular types of renal osteodystrophy are associated with either increased or decreased skeletal concentrations of trace elements. Because these patients are at risk for alterations of trace elements as well as for morbidity from skeletal disorders, we measured trace elements in bone of patients with end-stage renal disease.**

Methods: **We analyzed bone biopsies of 100 end-stage renal failure patients enrolled in a hemodialysis program. The trace metal contents of bone biopsies with histological features of either osteomalacia, adynamic bone disease, mixed lesion, normal histology, or hyperparathyroidism were compared with each other and with the trace metal contents of bone of subjects with normal renal function. Trace metals were measured by atomic absorption spectrometry.**

Results: **The concentrations of aluminum, chromium, and cadmium were increased in bone of end-stage renal failure patients. Comparing the trace metal/calcium ratio, significantly higher values were found for the bone chromium/calcium, aluminum/calcium, zinc/calcium, magnesium/calcium, and strontium/calcium ratios. Among types of renal osteodystrophy, increased bone aluminum, lead, and strontium concentrations and strontium/calcium and aluminum/calcium ratios were found in dialysis patients with osteomalacia vs the other types of renal osteodystrophy considered as one group. Moreover, the concentrations of several trace elements in bone were significantly correlated with each other. Bone aluminum was correlated with the time on dialysis, whereas bone iron, aluminum, magnesium, and strontium tended to be associated with patient age. Bone trace metal concentrations did not depend on vitamin D intake nor on the patients' gender.**

Conclusions: **The concentration of several trace elements in bone of end-stage renal failure patients is disturbed, and some of the trace metals under study might share pathways of absorption, distribution, and accumulation. The clinical significance of the increased/decreased concentrations of several trace elements other than aluminum in bone of dialysis patients deserves further investigation.**

In patients with uremia, trace element disturbances might occur because of (*a*) reduced renal function; (*b*) proteinuria, leading to losses of protein-bound elements; (*c*) alterations in gastrointestinal absorption because of alterations in, e.g., vitamin D metabolism; and (*d*) the dialysis procedure per se *(1, 2)*. Indeed, according to the concentration gradient between the ultrafiltrable amount of a particular element in serum and its concentration in the dialysis fluid, some trace elements may be removed, whereas others present as contaminants in the dialysis solution could be transferred to the patients.

In a survey on multielement analysis of bone, Zwanziger *(3)* showed that there are important discrepancies between the normal values of bone trace metal concentrations in various reports in the literature. These studies deal mainly with trace metal concentrations in bone of

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subjects with normal renal function. With the exception of aluminum and iron, little is known of the accumulation/deficiency of trace metals in bone of patients with end-stage renal failure treated by dialysis; this is rather surprising in view of the fact that these subjects represent a typical population at risk for alterations of these substances.

When present at increased concentrations, some trace metals may alter bone metabolism. In dialysis patients, the role of aluminum in the development of the low turnover osteodystrophic lesions, i.e., osteomalacia and adynamic bone, is now well recognized *(4, 5)*. Aside from aluminum, iron accumulation has, within the latter population, also been associated with the development of both osteomalacia *(6)* and adynamic bone disease *(7)*. In subjects with normal renal function, cadmium exposure has been related to a variety of bone lesions *(8)*, of which the so-called "itai-itai" disease is the most severe *(9)*. The present knowledge of the concentration in bone and the possible toxic effects on bone metabolism of other trace metals is rather fragmentary. Osteoporotic lesions have been associated with magnesium deficiency *(10)*, whereas in experimental studies, both zinc and copper were suggested to play a role in bone formation and resorption *(11–13)*. Strontium has been associated with the development of rachitis *(14)* and more recently with the development of osteomalacia in both rats and humans *(15, 16)*. Although some evidence for accumulation of chromium in dialysis patients has been presented *(17)*, data on the effect of the element on bone are scarce. In a recent study on the anabolic effects of insulin on bone, a role for chromium picolinate in the preservation of bone density was suggested *(18)*. Lead has been shown to alter bone turnover by either affecting calciotropic hormones or bone matrix synthesis *(19, 20)*.

Whether in dialysis patients the concentrations of trace elements in bone: (*a*) other than aluminum might also be disturbed; (*b*) may differ according to the type of renal osteodystrophy; or (*c*) correlate with each other, the aluminum, iron, copper, zinc, chromium, cadmium, lead, magnesium, strontium, and calcium content was determined in a large number of bone biopsies of dialysis patients presenting with the various types of renal osteodystrophy, as evidenced by histological and histomorphometric analysis and compared with the concentrations noted in subjects with normal renal function.

Patients and Methods

PATIENTS

Transiliac bone biopsies were taken in 100 end-stage renal failure patients treated by hemodialysis. In these subjects, bone biopsies were taken in the frame of previous studies of our group on adynamic bone disease *(21)* and the value of the low-dose desferrioxamine test in the diagnosis of aluminum-related bone disease *(22)*. Patients came from various centers of geographical areas all over the world: Belgium (n = 46), Greece (n = 39), Czechia (n = 7), Argentina (n = 3), and Egypt (n = 5). There were 48 males. Patients had a mean ± SD age of 58.8 ± 13.2 years and had been 5.2 ± 4.2 years in dialysis. Some of the patients (44.2%) received vitamin D. The indications for performing a bone biopsy were diverse: suspicion of aluminum intoxication, abnormal concentrations of intact parathyroid hormone (either high or low), diagnostic work-up before parathyroidectomy, a low bone alkaline phosphatase concentration, or suspicion or combination of any of these pathologies. Before biopsy, a double tetracyclin labeling was performed in the dialysis patients following a standardized procedure *(21)* to determine bone formation rate (BFR) and allow histological classification. Criteria for the different types of bone disease are outlined below. In addition, bone biopsies were also taken in 10 subjects with normal renal function.

BONE BIOPSY SAMPLING

Bone biopsies were taken under local anesthesia using a Bordier-Meunier needle *(21)*. During sampling and sample preparation, particular attention was paid to avoid contamination *(23, 24)*. Therefore, every item used from the moment of sampling until analysis was regarded as a potential source of contamination and checked to not contain or leach detectable amounts of any of the trace elements under study. The use of reagents for sample preparation was kept minimal and also checked to not contain any of the analytes of interest. Only plastic material was used for sample storage and sample preparation. Bone samples were divided in two pieces. One part was put in Burkhardt's solution and transferred to 700 mL/L ethanol after 24 h for storage at 4 °C until further processing for histological examination. The second part was weighed directly after sampling and stored at −20 °C until chemical analysis was performed.

HISTOLOGICAL BONE BIOPSY EXAMINATION

Undecalcified, glycol methacrylate-embedded, 4-μm thick bone sections were stained according to Goldner for descriptive histology. Bone histological data as well as dynamic parameters were reported according to the standardized nomenclature and definitions *(25)*. Different types of renal osteodystrophy were diagnosed according to the amount of osteoid, the presence of fibrosis, and the BFR: normal histology = osteoid area <12%, no fibrosis, and BFR 97–613 $\mu m^2/mm^2$ per day; hyperparathyroidism = osteoid area <12%, no fibrosis (mild), with fibrosis (osteitis fibrosis), and BFR >613 $\mu m^2/mm^2$ per day; osteomalacia = osteoid area >12%, no fibrosis, and BFR <97 $\mu m^2/mm^2$ per day; adynamic bone disease = osteoid area <12%, no fibrosis, and BFR <97 $\mu m^2/mm^2$ per day; and mixed lesion = osteoid area >12%, with fibrosis. Histological examinations of bone biopsies were performed at the UCLA School of Medicine (W.G.G.).

CHEMICAL BONE BIOPSY ANALYSIS

Before measurement, 20–500 mg wet weight transiliac bone biopsies were digested at 90 °C to 100 °C in 1–2 mL of nitric acid (Suprapur) in stoppered polytetrafluoroethylene test tubes for at least 3 h until a clear digest was obtained *(26)*. According to the sample weight, the clear digest was adjusted to either 10, 25, or 50 mL with doubly distilled water in polypropylene volumetric flasks and subsequently transferred to stoppered polystyrene test tubes for storage at −20 °C until analysis. Losses during the digestion procedure were checked for by calculating the recovery, i.e., comparing the concentration of analyte added to a bone matrix-matched solution after digestion vs the situation in which the element of interest had not undergone the digestion procedure. For each of the elements under study, the recovery was close to 100%, whereas within- and between-run imprecision was <10% *(23, 26–28)*. Magnesium and calcium were determined in the bone digestion liquid by flame atomic absorption spectrometry (FAAS; Perkin-Elmer Model 3110). Here, before FAAS analysis, samples were diluted 1:100 (magnesium) and 1:500 (calcium) in doubly distilled water to which 1 g/L lanthanum was added to limit phosphate interferences. Aluminum, copper, zinc, iron, lead, cadmium, strontium, and chromium were measured by means of electrothermal Zeeman atomic absorption spectrometry (Perkin-Elmer Spectrometer Model 3030; Graphite Furnace HGA 600). For each of the elements under study, optimal dilution factors, calibration methods, and instrumental conditions were either optimized or set according to methods published previously *(26–31)*. Matrix-matched calibration curves (addition calibration technique) were used for the determination of zinc, lead, aluminum, cadmium, and chromium, whereas iron, magnesium, calcium, strontium, and copper were measured against aqueous standards (direct calibration). Detection limits calculated for a 100-mg weighing bone biopsy contained in 25 mL of digestion liquid and using injection volumes of 5 μL (zinc), 10 μL (strontium, copper), or 20 μL (lead, cadmium, aluminum, iron, chromium) were 12.0 ng/g (cadmium), 6.7 μg/g (zinc, 20-fold dilution), 1.5 μg/g (lead), 0.15 μg/g (aluminum), 0.1 μg/g (chromium), 3.9 μg/g (iron, 10-fold dilution), 0.3 μg/g (copper), 2.0 μg/g (strontium, 40-fold dilution), 110 μg/g (magnesium, 100-fold dilution), 6.9 mg/g (calcium, 500-fold dilution). Linear working ranges for the determination of the various elements were 0–100 μg/L (iron), 0–60 μg/L (zinc), 0–75 μg/L (lead), 0–200 μg/L (aluminum), 0–30 μg/L (cadmium), 0–625 μg/L (magnesium), 0–5000 μg/L (calcium), 0–200 μg/L (copper), 0–10 μg/L (chromium), and 0–25 μg/L (strontium). Analytical accuracy of the calcium, magnesium, lead, zinc, strontium, and iron measurements was checked against the IAEA-H-5 Animal Bone Standard Reference Material (Table 1). No certified values are available for chromium, cadmium, copper, and aluminum measurement in bone.

Table 1. Accuracy of trace metal determination in bone.[a]

Element	Value found (± SD)[b]	Certified value (error, %)
Calcium	203 ± 15 mg/g	212 mg/g (3.8%)
Magnesium	3391 ± 188 μg/g	3550 μg/g (2.5%)
Lead	3.2 ± 0.4 μg/g	3.1 μg/g (18%)
Zinc	93 ± 11 μg/g	89 μg/g (5.9%)
Iron	72.1 ± 0.1 μg/g	79 μg/g (7.5%)
Strontium	95.9 ± 1.4 μg/g	96 μg/g (8.6%)

[a] Standard Reference Material IAEA-H-5 (animal bone). Certified values for aluminum, chromium, cadmium, and copper are not available in bone reference material.

[b] Values are the mean ± SD of four determinations.

All bone electrothermal atomic absorption spectrometry analyses were performed at the department of Nephrology-Hypertension of the University of Antwerp, whereas FAAS analyses were done at the department of Toxicology of the State University of Ghent (A.O.V.). Analyses were performed in single batches/element whenever possible. In case all samples could not be analyzed within one assay, several samples of the previous assay were included, which then served as internal controls. Because the concentration for several trace elements in bone may greatly depend on bone density, i.e., the proportion of cortical vs trabecular bone, data are also expressed as trace metal/calcium ratio; calcium is considered to be a measure of bone density.

BIOCHEMICAL ANALYSES

Serum intact parathyroid hormone concentrations were determined by means of a two-side immunoradiometric assay (Nichols Institute). For the measurement of serum osteocalcin, we used the radioimmunoassay from Incstar. Bone alkaline phosphatase was determined by an agarose gel electrophoretic method described by Van Hoof et al. *(32)*.

STATISTICS

Data are expressed as median (range) and/or mean ± SD. Relationships between bone trace element content vs age and vs time on dialysis were assessed by Spearman rank order correlation. Spearman rank order correlation was also used for assessing the interrelationship between the various elements under study. Because in total 49 correlations were made (Tables 3 and 4), Bonferroni adjustment was applied, and $P < 0.05{:}49$, i.e., <0.001, was considered significant at a two-tailed level. Differences in the percentage of patients taking vitamin D between the various groups of renal osteodystrophy were assessed by χ^2 analysis, followed by Bonferroni correction, considering P values significant when <0.005. The Mann–Whitney U-test, in combination with Bonferroni correction, was also used for pairwise comparison of the various biochemical parameters in the various types of renal osteodystrophy. Here, $P < 0.05{:}10$, i.e., <0.005, was considered significant at a two-tailed level. Comparison of the bone trace metal content in renal failure patients vs normal renal function

Table 2. Biochemical and clinical data of the study groups presenting the various types of renal osteodystrophy.[a]

Bone lesion	n	Age, years	Time in dialysis, years	Sex, M/F	Vitamin D intake, %	iPTH,[b] ng/L	BAP, U/L	OC, μg/L
ABD	35	61.0 (41.0–86.0)	2.6 (0.1–13.0)[c]	18/17	41	90 (1–367)	20 (9–636)[d]	7.3 (1.5–20.8)[d]
		(62.4 ± 9.2)	(3.7 ± 3.3)			(122 ± 102)	(48 ± 116)	(7.9 ± 4.8)
MX	21	64.5 (27.0–75.0)	3.8 (0.1–24.0)	8/13	45	353 (91–1152)[e]	125 (22–1919)[f]	17.1 (3.2–34.3)
		(60.0 ± 12.4)	(5.5 ± 5.6)			(425 ± 292)	(347 ± 567)	(16.1 ± 7.1)
N	13	64.5 (44.0–94.0)	5.5 (0.5–11.0)	5/8	31	200 (31–6560)	35 (12–119)	10.0 (6.9–21.3)
		(64.3 ± 12.8)	(5.7 ± 3.0)			(212 ± 164)	(41 ± 32)	(11.4 ± 4.5)
HPTH	21	56 (18.0–72.0)	6.8 (0.7–18.0)	11/10	29	797 (180–2240)[g]	185 (30–1604)[f]	19.0 (5.6–58.3)[f]
		(51.2 ± 15.9)	(7.3 ± 4.2)			(888 ± 512)	(288 ± 381)	(21.8 ± 12.1)
OM	10	48.5 (41–76)	4.5 (0.8–7.0)	6/4	70	109 (8–318)	50 (11–715)	11.5 (3.4–23.1)
		(53.0 ± 12.9)	(4.1 ± 2.4)			(110 ± 102)	(160 ± 248)	(11.5 ± 6.5)

[a] Data are expressed as median (range) and (mean ± SD).

[b] iPTH, intact parathyroid hormone; BAP, bone alkaline phosphatase; OC, osteocalcin; ABD, adynamic bone disease; MX, mixed bone lesion; N, normal histology; HPTH, hyperthyroidism; OM, osteomalacia.

[c] $P < 0.005$ vs HPTH.

[d] $P < 0.005$ vs MX and HPTH.

[e] $P < 0.005$ vs ABD, HPTH, and OM.

[f] $P < 0.005$ vs ABD and N.

[g] $P < 0.005$ vs ABD, MX, N, and OM.

was done using the Mann–Whitney *U*-test. Bonferroni correction was applied because comparisons were made for the 10 metals under study. $P < 0.05{:}10$, i.e., < 0.005, was considered significant. To check whether particular types of renal osteodystrophy were associated with alterations in bone metal content–trace metal/calcium ratio, the various types were compared with all other types taken as one group. For those in which a difference was noted (i.e., aluminum, aluminum/calcium, lead, strontium, and strontium/calcium in osteomalacia), a post hoc comparison of that particular bone lesion with all other types (n = 4) was done using the Mann–Whitney *U*-test with Bonferroni correction. Here, differences were considered significant provided a *P* threshold of $< 0.05{:}4$, i.e., < 0.0125, was achieved.

Bone analyte concentrations were expressed in μg/g wet weight. The analyte/calcium ratio was calculated to allow correction for bone density *(33, 34)*.

Results

Histological examination of the bone biopsies of 100 hemodialysis patients revealed the presence of hyperparathyroidism in 21 of them; 10 subjects had osteomalacia; adynamic bone disease was found in 35 individuals; 21 were classified as having mixed lesion; and 13 patients had a normal bone histology. Subjects with normal renal function had normal bone histology. Clinical and biochemical data of the groups presenting the various types of renal osteodystrophy are presented in Table 2.

Several elements correlated with age. Bone iron (r_s = 0.26; $P < 0.05$), iron/calcium ratio (r_s = 0.25; $P < 0.05$) and lead/calcium ratio (r_s = 0.21; $P < 0.05$) tended to increase with age, whereas bone aluminum ($r_s = -0.30$; $P < 0.05$), aluminum/calcium ($r_s = -0.27$; $P < 0.05$), bone chromium ($r_s = -0.21$; $P < 0.05$), magnesium/calcium ($r_s = -0.24$; $P < 0.05$), bone strontium ($r_s = -0.28$; $P < 0.05$), and strontium/calcium ($r_s = -0.29$; $P < 0.05$) decreased with age. With the exception of bone aluminum (r_s = 0.45; P = 0.001) and the bone aluminum/calcium ratio (r_s = 0.44; $P < 0.001$), none of the elements under study correlated with the time patients were on dialysis. No relationship was found between the bone trace metal content or trace metal/calcium ratio and sex, or vitamin D intake.

Significant ($P < 0.001$) correlations were found between

Table 3. Spearman correlation matrix of the various bone trace element concentrations in 100 hemodialysis patients.

	Iron	Lead	Copper	Chromium	Aluminum	Cadmium	Zinc	Magnesium	Strontium	Calcium
Iron	1.00									
Lead	−0.174	1.00								
Copper	0.093	−0.027	1.00							
Chromium	0.064	0.062	0.320	1.00						
Aluminum	−0.098	0.131	−0.080	0.038	1.00					
Cadmium	0.174	−0.030	0.516[a]	0.121	0.028	1.00				
Zinc	−0.042	0.488[a]	0.245	0.516[a]	−0.002	0.155	1.00			
Magnesium	−0.189	0.349	0.029	0.243	0.034	−0.066	0.454[a]	1.00		
Strontium	−0.361	0.413	0.140	0.418[a]	−0.038	−0.135	0.510[a]	0.574	1.00	
Calcium	−0.080	0.507[a]	0.059	0.309	−0.135	−0.047	0.576[a]	0.723[a]	0.703[a]	1.00

[a] $P < 0.001$.

the bone calcium content and the bone strontium (r_s = 0.70), magnesium (r_s = 0.72), lead (r_s = 0.51), and zinc (r_s = 0.58) content. These correlations with calcium indirectly indicate that these elements must accumulate mainly in cortical or compact bone. In view of the above, the relationship also noted between lead and zinc (r_s = 0.49; P <0.001; Table 3) could more or less be expected. On the other hand, in the absence of any correlation with bone calcium, cadmium and copper were significantly correlated with each other (r_s = 0.52; P <0.001; Table 3), as were the calcium ratios of both elements (r_s = 0.66; P <0.001; Table 4). Also, the bone chromium and bone zinc concentrations were found to be significantly correlated (r_s = 0.52; P <0.001). For several elements, no correlation was found when absolute concentrations were considered, whereas a significant relationship was noted for the trace metal/calcium ratio.

By comparing the trace element contents in bone of end-stage renal failure patients vs those in subjects with normal renal function, bone aluminum (P <0.00001) and chromium contents (P = 0.0004) were markedly increased. Also, when correction was made for bone density, i.e., data were expressed as the trace metal/calcium ratio, significant differences still existed for these elements and also for the zinc/calcium, magnesium/calcium, and strontium/calcium ratios (Fig. 1).

When comparing the different types of renal osteodystrophy, no significant differences were observed in the bone calcium content. With the exception of osteomalacia, the trace element content of bone biopsies in the different types of renal osteodystrophy did not differ significantly from each other. However, by comparing the bone trace element concentrations in patients with osteomalacia with those observed in the other types of renal osteodystrophy taken as one group, a significant increase was noted in the bone lead (P = 0.016), aluminum (P = 0.023), aluminum/calcium (P = 0.03), strontium (P = 0.006), and strontium/calcium (P = 0.0008) content. Post hoc pairwise comparison of the concentration of these elements in osteomalacia vs the other types of renal osteodystrophy taken as separate groups revealed that only the bone strontium content and bone strontium/calcium ratio were significantly increased vs all other types of renal osteodystrophy (P <0.0125).

Discussion

Numerous anomalies are observed when reading through the literature dealing with trace metal concentrations in bone. Not all of the published reports present data on the reliability of the analytical method used to measure the trace metal content in bone, which often makes interpretation and comparison of the data presented difficult. Moreover, a variety of techniques have been used for trace metal determination in bone, which with particular regard to the older techniques do not always meet the minimal requirements of analytical performance. Also, the trace element content in biological samples may be subject to biological variations (genetic disorders, age, renal function, and others), postmortem changes, and regional discrepancies *(3)*.

Results presented in this report are based on sensitive and accurate AAS methodologies. Samples are decomposed by simple wet ashing in nitric acid. Compared with dry ashing and extraction procedures *(35)*, this technique is rapid and in general provides better recoveries. We used fresh bone samples and expressed our data in μg/g wet weight. This condition (*a*) best reflects the in vivo situation; (*b*) does not require a time-consuming drying procedure, which is often the rate-limiting step in the analytical procedure; and (*c*) because of the limited number of manipulations, is much less prone to contamination. Wet weight measurements, however, require fresh tissue to ensure correct sample weighing. In cases where this was not available, some authors have made documentation of trace element concentrations in "fresh" tissue possible by using more readily available formalin-fixed samples, which when used under contamination-free conditions, appear not to influence the concentration of most elements *(36)*. Others applied conversion factors to calculate and compare trace element concentrations in fresh vs dried and ashed bone samples *(3)*. Here, as a general rule, the conversion of 10 g of fresh = 6 g dry = 3 g ash should be used *(3)*.

A major problem inherent to comparative studies on

Table 4. Spearman correlation matrix of the various trace elements/calcium ratios in bone of 100 hemodialysis patients.

	Iron/ Calcium	Lead/ Calcium	Copper/ Calcium	Chromium/ Calcium	Aluminum/ Calcium	Cadmium/ Calcium	Zinc/ Calcium	Magnesium/ Calcium	Strontium/ Calcium
Iron/Calcium	1.00								
Lead/Calcium	−0.046	1.00							
Copper/Calcium	0.350	0.047	1.00						
Chromium/Calcium	0.183	−0.082	0.423[a]	1.00					
Aluminum/Calcium	0.151	0.283	0.143	0.149	1.00				
Cadmium/Calcium	0.364	0.085	0.657[a]	0.225	0.185	1.00			
Zinc/Calcium	0.278	0.286	0.450[a]	0.393	0.268	0.399	1.00		
Magnesium/Calcium	0.120	0.054	0.200	0.235	0.404[a]	0.177	0.314	1.00	
Strontium/Calcium	−0.391	0.021	0.049	0.244	−0.012	−0.133	0.049	0.157	1.00

[a] P <0.001.

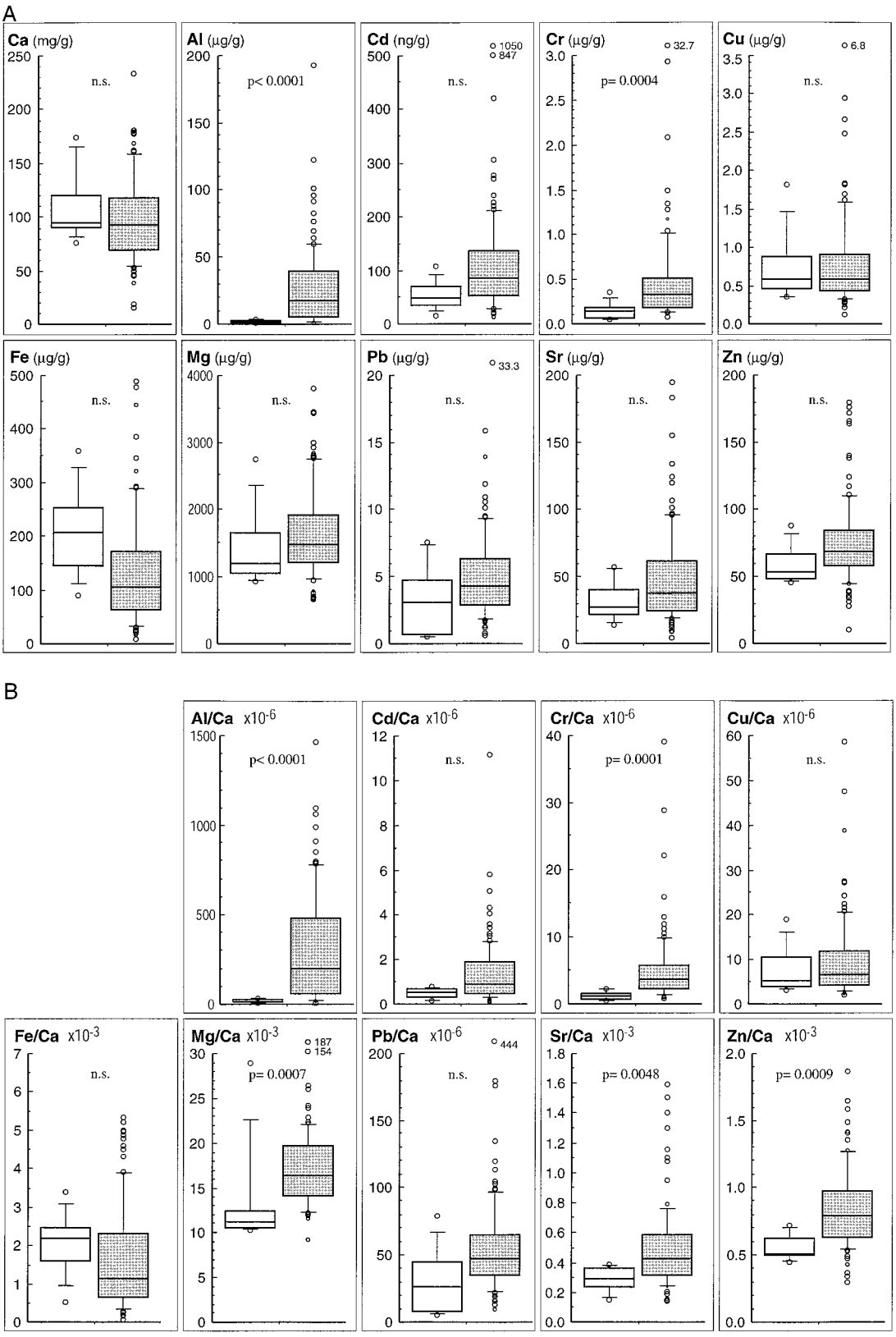

Fig. 1. Box-whisker plots of trace metal concentration (*A*) and trace metal/calcium ratio (*B*) in bone of dialysis patients (■; n = 100) vs subjects with normal renal function (□; n = 10).

P <0.005 was considered significant. n.s., not significant.

bone trace metal concentrations is differences or changes in tissue composition. Sample heterogeneity can lead to serious errors, especially if the analyte is not homogeneously distributed within the bone tissue, e.g., cortical vs trabecular bone, compact vs spongeous bone, or calcified vs noncalcified bone. This has been addressed previously by Van de Vyver et al. *(33)* studying bone lead in dialysis patients. In lead poisoning, the element is predominantly enriched in the compact regions of bone. Therefore, when expressing data in absolute concentrations, great differences were noted between tibial (mainly compact or highly calcified) and transiliacal (mainly spongeous, less calcified) bone within a single individual. When the metal/calcium ratio was calculated, however, differences were no longer present, indicating the bone trace metal/calcium ratio, to a certain extent, is able to correct for sample heterogeneity. To overcome variations in the relative proportions of cortical bone, trabecular bone, and marrow from biopsy to biopsy, leading to substantial variations in the concentration of those metals being heterogeneously distributed within bone, the metal/calcium ratio was also determined in the present work.

Data presented in the present report were obtained in a representative dialysis population recruited from various geographical areas from all over the world. The prevalence of the various bone lesions agrees well with the present spectrum of renal osteodystrophy. Results clearly indicate that the concentration of several trace elements is disturbed in end-stage renal failure patients. Among these, the accumulation of aluminum in dialysis patients originating from the intake of aluminum-containing phosphate binders and the use of aluminum-contaminated dialysis fluids is now well recognized *(4, 5)*. However, the present knowledge on the status of trace elements other than aluminum is limited, particularly for what causes their concentration in the bone compartment which, as is known for aluminum *(37)*, is an important storage organ for other trace elements *(34, 38)*.

We found that besides aluminum, the bone chromium concentration was also increased in the dialysis population. After correcting for bone density by calculating the calcium ratio, significant increases were also found for zinc, magnesium, and strontium. In contrast to a recent report by Navarro et al. *(39)*, no loss of bone calcium was noted in the dialysis population under study when compared with subjects with normal renal function or for any of the various types of renal osteodystrophy.

It cannot be deduced from the present data whether the increased concentrations are a result of disturbances in the gastrointestinal absorption resulting from an altered vitamin D metabolism *(40)*, the dialysis treatment itself *(41)*, or the absence of efficient renal excretion. Accumulation of aluminum via the oral intake of aluminum-containing phosphate binders or the use of contaminated dialysis fluids is well known. In this context, the correlation of bone aluminum with the time on dialysis is not surprising.

Although to the best of our knowledge no data on the accumulation of chromium in bone of dialysis patients have been published thus far, increased serum chromium concentrations have been reported in these subjects, which seemed to originate from the use of contaminated dialysis fluids *(42)*. In the present study, increased accumulation of chromium in bone could not be associated with the development of a particular type of renal osteodystrophy. Aside from bone, it is worth mentioning that soft tissue retention of aluminum and chromium has also been reported previously in renal failure *(43)*.

Increased bone strontium concentrations were found in dialysis patients with osteomalacia. Recent data indicate that the use of strontium-contaminated dialysates may put particular dialysis centers at an increased risk of accumulation of the element *(44)*. Although further studies are required, evidence for a causative role of the element in the development of this particular bone disease has been provided recently in an experimental rat study *(15)*.

Aside from aluminum and strontium, osteomalacia was also accompanied by increased bone lead concentrations. In previous studies, we showed that chronic renal failure or dialysis treatment per se does not lead to bone lead accumulation *(33)*. In view of this and notwithstanding that some pathology of lead on the skeleton has been hypothesized *(19)*, increased bone lead concentrations most probably occur secondary to the presence of osteomalacia. In this respect, the notion that lead tends to accumulate mainly at the bone surface is of particular interest. Indeed, in contrast to an intact bone metabolism, removal of the element from this site by deposition of new material *(45)* occurs at a much slower rate in the presence of osteomalacia.

Confirming previous data by Smythe et al. *(2)*, we also found bone zinc concentrations to be increased in dialysis patients. Although some discrepancies still exist, literature data suggest that the dialysis treatment itself has little or no effect on serum zinc concentrations. Earlier observations indicating serum zinc concentrations in dialysis patients to be decreased *(46)* may also point toward translocation of the element in uremia *(1)*.

In the present study, several other interesting associations were noted. Whereas bone strontium and magnesium were inversely correlated with age, bone calcium was not. Also, bone aluminum decreased with age. Whether these observations are because of age-dependent alterations in gastrointestinal absorption or bone resorption need further investigation.

As could be expected, bone magnesium, bone strontium, and bone calcium were closely correlated with each other. Considering bone calcium content as a measure of bone density, the positive correlation of lead and zinc with the latter element indicates that these trace metals are mainly localized in compact bone. This may also explain, at least in part, the interrelationships noted between the bone zinc and lead concentrations.

Interestingly, we also noted a strong relationship between the bone copper and bone cadmium concentrations. The fact that trace metals were not correlated with bone calcium or with strontium or magnesium suggests that these elements are diffusely distributed in both cortical and trabecular bone. This and the highly significant correlation that we noted when the calcium ratios of these elements were considered further indicate that both of these elements may share some common absorption, transport, and tissue distribution pathways. To what extent their similar ionic radii (both 0.92Å) might also play a role in this relationship is worth considering as well. Together with further investigations on the clinical relevance of disturbed bone trace element concentrations in end-stage renal failure patients, these findings may offer interesting perspectives for future studies.

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