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The Reaction of Different Skeletal Sites to Metabolic Bone Disease – A Micromorphometric Study*

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Histologische Veränderungen an verschiedenen Skelettorten bei systemischen Knochenerkrankungen – eine mikromorphometrische Untersuchung

Zusammenfassung. Bei skelettgesunden Kontrollpersonen ($n=8$), bei Patienten mit chronischer Niereninsuffizienz ($n=13$) und bei Patienten mit malignen Tumoren ($n=11$) wurden post mortem Knochenbiopsie-Proben aus dem 5. Lendenwirbelkörper, aus dem Beckenkamm, aus dem Femurkopf und aus dem interepikondylären Teil des distalen Femur entnommen. Unentkalkte, nach Masson-Goldner gefärbte Dünnschnitte wurden histomorphometrisch untersucht. Die Varianz einzelner histomorphometrischer Parameter zwischen den unterschiedlichen Skelettorten wurde durch Varianzanalyse überprüft.

Die histomorphometrischen Parameter, welche in Beziehung zum inneren Knochenumbau stehen, waren in den einzelnen Skelettorten außerordentlich unterschiedlich. Die lokalen Umbauraten differieren daher wahrscheinlich stark (Beckenkamm > Lendenwirbelkörper > Femurkopf > distaler Femur). Bei Patienten mit Knochenerkrankungen änderten sich alle histomorphometrischen Parameter gleichsinnig an allen untersuchten Skelettorten. Lediglich die Volumendichte und die Oberflächendichte inaktiven Osteoids zeigte in den einzelnen Skelettorten ein signifikant unterschiedliches Verhalten.

Die Untersuchung belegt, daß bei systemischen Knochenerkrankungen lokale Faktoren nicht die Antwort des Skeletts auf systemische Stimuli beeinträchtigen. Die Messergebnisse belegen, daß Knochenbiopsie-Proben aus der Beckenkammspongiosa mit hinreichender Genauigkeit die Vorgänge widerspiegeln, die in anderen Skelettorten ablaufen. Lediglich für inaktives Osteoid konnte bei urämischen Patienten gezeigt werden, daß in der Beckenkammspongiosa eine – im Vergleich zu Skelettorten mit niedrigerer Umbaugeschwindigkeit – überproportioniert starke Akumulation stattfindet.

Schlüsselwörter: Knochenhistologie urämischer Osteopathie – Calciumstoffwechsel – metabolische Knochenerkrankungen – Mikromorphometrie.

Summary. It is unkown whether histomorphometric parameters of bone surface remodelling change uniformly throughout the skeleton in metabolic bone disease.

In patients without skeletal disease ($n=8$), in patients with chronic renal failure ($n=13$) and in patients with malignant tumors ($n=11$), post mortem bone samples were taken from the core of the 5th lumbar vertebra, from the iliac crest, from the femural head and from the interepicondylar portion of the distal femur. Histomorphometric analysis was carried out in undecalcified sections stained after Masson-Goldner. The dispersion of histomorphometric parameters between the different skeletal sites (cancellous bone) was evaluated by analysis of variance.

There existed marked differences in the surface extension of the histomorphometric parameters which are related to bone remodelling, suggesting marked differences in the local remodelling rates (iliac crest > lumbar vertebra > femural head > distal femur).

In patients with bone disease all histomorphometric parameters changed uniformly throughout the skeleton with the exception of inactive osteoid.

The study shows that in metabolic bone disease local factors do not interfere with remodelling in response to systemic factors. It also provides evidence that bone biopsy samples from iliac crest spongiosa reflect with reasonable accuracy the changes occurring elsewhere in the skeleton, although inactive osteoid may accumulate in the iliac crest out of proportion to its increase in low-turnover sites.

Key words: Bone – Metabolic Bone Disease – Uremic Osteodystrophy – Calcium Metabolism – Malignant Tumors.

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Bone must not be envisaged as a homogenous organ responding to hormonal or metabolic challenges in an uniform fashion like a parenchymatous organ. Much to the contrary, it is composed of a heterogenous population of structural units (osteons) of different age and geometry and exposed to different mechanical strain [1]. The magnitude of the response (e.g. the number of remodelling units) in a given volume of bone depends, among other things, on the basal rate of remodelling at the respective site and possibly also on the local pool of osteoprogenitor cells. Remodelling rates in normal individuals are presumed to differ in different bones or in different sites of the same bone [2, 3]. They are for instance lower in the diaphysis than in the metaphysis which explains the early appearance of intracortical changes in the metaphysis of uremic patients, while diaphyseal changes occur late or not at all [4].

It was the purpose of the present investigation to study to what extent remodelling parameters differ between several skeletal sites. It was also investigated whether the relative differences in remodelling parameters between several skeletal sites are maintained in metabolic bone disease or whether these skeletal sites respond to systemic stimuli to a different extent. Such homogeneity of response, or lack of it, is relevant to the question of whether bone biopsy samples accurately reflect changes occurring elsewhere in the skeleton.

This problem was investigated by histomorphometric analysis of several bone samples taken from patients without skeletal disease, patients with chronic renal failure and patients with malignant tumors. The variance between the different skeletal sites within individual patients was evaluated by analysis of variance.

The results document that in metabolic bone disease the change in histomorphometric remodelling parameters is quite uniform.

Materials and Methods

Three groups of patients were studied:

1. group: control patients without skeletal disease ($n=8$): 4 males, 4 females; mean age 41.3 years (range 17–50). The causes of death were traffic accident ($n=5$), anaphylactic shock ($n=1$), sudden death in a patient with a history of hypertension ($n=1$), pulmonary embolism after hysterectomy for uterine fibroma ($n=1$).

2. group: patients with chronic renal insufficiency ($n=13$): 9 males, 4 females; mean age 52 years (range 29–71); 2 patients had been on hemodialysis. The underlying renal disease was glomerulonephritis ($n=5$), polycystic disease ($n=2$), pyelonephritis ($n=2$), chronic ureteric obstruction ($n=2$), gout ($n=1$) and Alport's disease ($n=1$).

3. group: patients with malignant tumors and tumor cachexia ($n=11$): 9 males, 2 females; mean age 59.7 years (range 29–77); the patients had bronchial carcinoma ($n=7$), renal cell carcinoma, gastric carcinoma, prostatic cancer and Brill-Symmer's lymphoma (1 patient each). All patients had been immobilised for prolonged periods of time. None of the patients was hypercalcemic. Most of the patients had skeletal metastases; all biopsy samples were free of metastases, however, as verified by microscopical examination.

Bone samples (cubes $1 \times 1 \times 1$ cm in size) were obtained at autopsy from 4 skeletal sites:

1. 5th lumbar vertebra (core of the vertebral body)
2. iliac crest (1 cm behind the ant. sup. spine and evaluated 0.8 cm underneath the cortical surface)
3. femural head (right side) 1 cm underneath the articular surface parallel to the direction of the femural neck
4. distal femur (right side); in the middle between the two epicondyli, 1 cm underneath the articular cartilage.

Undecalcified sections (5u) were stained after Masson Goldner [5] and analysed micromorphometrically as described by Merz and Schenk [6]. The measurements were made at a magnification of $\times 100$ with an eye-piece carrying a special grid (Firma Wild, Herrbrugg/Switzerland). Areas were measured by point counting and perimeter lengths by counting intersections using a grid with semicircular sampling lines. Histomorphometric parameters were calculated as described by Schenk [5].

Analysis of variance with equal replicates (ANOVA) was carried out, using the point counts and the intersection counts. The variance between skeletal sites within the individual patients was compared using the F-Test. The F-ratio was calculated as the ratio of the mean squares (controls/uremia or controls/tumor) which resulted from differences between the 4 skeletal sites (3 DF) as the source of variance. The Scheffé-Test was used to test the O-hypothesis that the variance between the different skeletal sites was no greater in patients with uremia or tumor cachexia than in control individuals without skeletal disease.

Results

The *histomorphometric parameters* are given in Table 1. With the exception of S_V and $S_{V_{ob}}$, which are somewhat lower in this series, the control values are in good agreement with the values previously reported by Schenk [6], Delling [7, 8] and Malluche [9, 10, 11].

In *control patients* bone density (V_V) was lower in the vertebral body and higher in the femural head as compared to iliac crest. Bone density was very low in the distal femur. Surface remodelling parameters and bone density did not change in parallel. The fraction of the trabecular surface covered by active ($S_{V_{ob}}$) or inactive ($S_{V_{oi}}$) osteoid seams or active Howship's lacunae ($S_{V_{hl}}$) was highest in iliac bone, lower in the vertebral body and still lower in the femural head or in the distal femur.

In *patients with malignant tumors* histomorphometric parameters were not significantly different.

In *patients with uremia* there was a significant increase in total osteoid ($S_{V_{os}}$), entirely accounted for by the increase in inactive osteoid ($S_{V_{oi}}$), and an increase in osteoclastic surface resorption ($S_{V_{hl}}$). There

Table 1. Histomorphometric Parameters

	V_V (mm³/cm³)				V_{Vos} (mm³/cm³)				S/V (mm²/mm³)				S_V (mm²/cm³)				S_{Vhem} (mm²/cm³) (%)				S_{Vos} (mm²/cm³) (%)				S_{Vob} (mm²/cm³) (%)				S_{Voi} (mm²/cm³) (%)				S_{Vhl} (mm²/cm³) (%)			
	V	I	FP	FD	V	I	FP	FD	V	I	FP	FD	V	I	FP	FD	V	I	FP	FD	V	I	FP	FD	V	I	FP	FD	V	I	FP	FD	V	I	FP	FD
CONTROLS	172	218	321	189	1,39	2,22	1,39	0	18,33	18,6	11,16	18,88	1998	2637	2316	2050	145	148	28,4	0,43	81,7	167	49,7	46,2	8,88	26,6	0,00	0,00	76,2	136	49,7	46,2	8,88	26,4	8,88	0,00
	±54,4	±62,7	±62,7	±90,8	±3,05	±5,0	±2,22	±0	±4,41	±3,31	±2,07	±7,85	±473	±493	±243	±243	±51,4	±50	±35	±1,14	±120	±182	±60,3	±51,3	±25,1	±47,7	±0	±0	±106,9	±144	±60,3	±51,3	±13,0	±23,4	±15,06	±0
(n = 8)																	7,25	5,57	1,23	0,02	3,97	6,20	2,08	2,69	0,41	0,86	0,00	0,00	3,57	5,34	2,08	2,69	0,38	1,10	0,39	0,00
																	±2,57	±1,90	±1,51	±0,05	±5,66	±6,35	±2,46	±3,36	±1,15	±1,50	±0	±0	±4,75	±5,53	±2,46	±3,36	±0,54	±1,09	±0,63	±0
UREMIA	124	179	288	151	0,99	13,3	4,44	0,89	19,8	18,6	14,1	18,6	1698	2256	2398	1699	118	151	9,51	0,43	311	562	245	109	9,83	10,9	25,1	13,1	368	550	220	96,0	25,1	67,7	55,7	27,3
	±30,5	±46,1	±111	±64,7	±15,3	±21,6	±7,22	±1,67	±4,27	±4,1	±5,9	±5,10	±306	±355	±418	±426	±41,7	±44,9	±16,9	±1,28	±406	±683	±280	±116	±19,56	±21,8	±49,6	±47,3	±401	±682	±245	±85,0	±25,4	±41,5	±64,2	±34,52
(n = 13)																	6,95	6,69	0,40	0,003	22,10	24,82	10,89	7,73	0,32	0,47	1,18	1,38	21,55	24,35	9,70	6,35	1,87	4,35	3,31	1,85
																	±2,46	±1,90	±0,70	±0,075	±22,29 *	±29,64 *	±12,84	±11,35	±0,81	±0,92	±2,35	±4,97	±22,17 *	±29,59 *	±11,06	±6,76	±1,74	±4,22 *	±4,55	±2,18
TUMOR	130	194	319	152	0,56	1,39	0,83	5,56	16,5	16,6	11,3	20,3	1532	2306	2359	1938	103	119	38,1	8,09	86,5	152	76,2	49	1,29	11,6	1,29	0,00	85,2	150	40,0	49	16,0	18,1	6,45	6,45
	±32,2	±65,2	±56,1	±36,6	±1,67	±2,22	±1,67	±1,11	±4,27	±3,45	±1,52	±4,82	±270	±485	±401	±241	±46,4	±60,6	±88	±22,0	±86,7	±198	±120	±38,3	±4,28	±20,9	±4,28	±0	±85,7	±180	±32,9	±38,3	±23,0	±38,06	±9,76	±7,42
(n = 11)																	6,72	5,16	1,62	0,42	5,90	6,48	1,70	2,53	0,07	0,42	0,05	0,00	5,83	6,06	1,64	2,43	1,06	0,85	0,27	0,33
																	±3,03	±2,63	±3,73	±0,11	±6,19	±5,92	±1,41	±1,86	±0,23	±0,72	±0,17	±0	±6,16	±5,66	±1,45	±1,85	±1,55	±0,82	±0,39	±0,39

V_V = volume density (mm^3/cm^3)

$V_{V_{os}}$ = volume density of osteoid (mm^3/cm^3)

S/V = specific surface, i.e. ratio of trabecular surface/trabecular volume (mm^2/mm^3)

S_V = surface density (mm^2/cm^3)

$S_{V_{hem}}$ = surface density of bone matrix – hematopoietic marrow interface (mm^2/cm^3) $\left(\frac{S_{V_{hem}}}{S_V} \times 100\,(\%)\right)$

$S_{V_{os}}$ = surface density of osteoid seams (mm^2/cm^3) (osteoid seams as percent of trabecular surface (%) $= \frac{S_{V_{os}}}{S_V} \times 100\Big)$

$S_{V_{ob}}$ = surface density of osteoblast-osteoid interface (mm^2/cm^3) $\left(\frac{S_{V_{ob}}}{S_V} \times 100\right)$

$S_{V_{oi}}$ = surface density of inactive osteoid (mm^2/cm^3) $\left(\frac{S_{V_{oi}}}{S_V} \times 100\right)$

$S_{V_{hl}}$ = surface density of bone-osteoblast interface (mm^2/cm^3) (bone of osteoclast as percent of mineralized trabecular surface) $= \frac{S_{V_{hl}}}{S_V - S_{V_{os}}} \times 100\Big)$

Biopsy Sites

V = 5th lumbar vertebra

I = iliac crest spongiosa

FP = proximal femur (femoral head)

FD = distal femur (interepicondylar portion)

* difference with control group p 0.05 (Wilcoxon's test for randomized samples)

Table 2. Variance between different sites in the skeleton of patients with uremia and patients with malignant tumors as compared with the variance between different skeletal sites in patients without skeletal disease

Parameter	F-value[a]	
	Uremia ($n=13$)	Tumor ($n=11$)
Bone density (hits)	1.88	2.19
Osteoid density (hits)	67.19[b]	4.39
Bone-marrow interface (random intersections)	2.55	2.35
Bone-hematopoietic marrow interface (random intersections)	1.27	1.68
Bone-active osteoid interface (random intersections)	1.95	3.90
Bone-inactive osteoid interface (random intersections)	34.08[b]	1.95
Bone-osteoclast interface (random intersections)	1.71	9.58[b]

[a] Analysis of variance with equal replicates (ANOVA); F = ratio of mean squares [source of variance: differences between 4 skeletal sites (vertebra, iliac crest, proximal femur, distal femur)]. F-Test (DF 3)

[b] $p<0.05$

was no change in bone matrix – hemotopoietic marrow-interface ($S_{V_{hem}}$). For unknown reasons, the characteristic increase in bone density which is usually found in patients with renal failure [12, 13] was not demonstrable in this study.

The *variance* between *different sites in the skeleton* which is a measure of skeletal heterogeneity, was compared between patients with uremia (or patients with malignant tumors) on the one hand and patients without skeletal disease on the other hand (Table 2). For none of the histomorphometric parameters (with the possible exception of bone-osteoclast intersection counts which showed borderline significance) was the variance between several skeletal sites different in patients with malignant tumors as compared with control patients.

For osteoid counts (from which $V_{V_{os}}$ is calculated) and bone-inactive osteoid intersections (from which $S_{V_{oi}}$ is calculated), the variance between the 4 skeletal sites found in uremic patients, differed significantly from the variance found in control patients. This indicates that for $V_{V_{os}}$ and $S_{V_{oi}}$ the relative increase at the 4 skeletal sites studied is not equal. This finding implies that the accumulation of inactive osteoid is not homogeneous throughout the skeleton. For none of the other histomorphometric parameters studied was the variance between the different skeletal sites significantly different in uremic patients.

Discussion

Several authors emphasized [12, 13, 14] that in metabolic bone disease one may note striking differences of reaction between different skeletal sites, viz. a decrease in cortical bone in the presence of an increase of spongy bone mass or metaphyseal sclerosis in the presence of rarification of diaphyseal bone. In patients with renal failure, Cohn et al. [13] found a poor correlation between total body calcium, measured by neutron activation analysis, and bone mineral content in the forearm, measured by the photon absorption technique. This was attributed to internal redistribution of bone mineral which was thought to result from the non-uniformity of the skeletal reaction. Similar discrepancies were noted in our experimental study in ovariectomised rats in which osteopenia in the metaphysis was observed in the presence of an increase of the width of cortical bone [15].

The present investigation documents striking differences in the surface extension of the histomorphometric parameters related to bone remodelling. To the extent that bone surface measurements reflect bone remodelling rates, these measurements would indicate that cancellous bone remodelling is highest in the iliac crest, followed by vertebra, femural head and distal femural metaphysis. The local remodelling activity per unit volume of bone is quite independent of the respective bone density, for the latter is highest in the femural head followed by iliac crest, distal femural metaphysis and vertebra. The rather low remodelling rate in the femural head of the adult skeleton is remarkable in view of its high rate in the growing skeleton which is one of the factors predisposing to epiphyseal slipping as indicated by our previous studies [16]. The reasons for the local differences in remodelling rate are unknown. Such differences of remodelling activity per unit volume bone may be related to differences of the surface/volume-ratio [17], differences of bone marrow capillary density [18] with concomitant differences in the availability of bone precursor cells, or differences of mechanical stress. The various bones studied were subjected to different mechanical stress, e.g. more static loading in the femural head and more shearing stress in the iliac crest resulting from the cinch strap mechanism of abdominal muscles. The importance of mechanical factors is illustrated by our previous study which indicated that the responsiveness of bone to PTH is altered by mechanical loading [19, 20].

The above measurements also demonstrate notable differences in the fraction of trabecular surface which is in contact with hematopoietic marrow ($S_{V_{hem}}$). This fraction of the trabecular surface may be involved in providing capillaries and thus osteoprogen-

itor cells. However, both the surface extension and the variance for this parameter were not appreciably different in patients with renal insufficiency or malignant disease.

The present investigation documents that the skeletal changes are quite uniform in cancellous bone of patients with uremic osteodystrophy. This applies to structural parameters (V_V, S_V, S/V) as well as to surface parameters which are related to bone remodelling and reflect cellular activities in remodelling sites ($S_{V_{ob}}$, $S_{V_{hi}}$). The statistical procedure chosen in this study permits to compare the spatial dispersion of histomorphometric parameters in uremic patients and in control patients. As indicated by this procedure the accumulation of inactive osteoid [measured both as osteoid density ($V_{V_{os}}$) and as extension of inactive osteoid seams ($S_{V_{oi}}$)] was non-uniform, i.e. inactive osteoid accumulated to a greater extent in sites with high local remodelling rates (iliac crest, lumbar vertebra) than in sites with low rates (femural head, distal femur).

These findings indicate that iliac crest bone biopsies adequately reflect what is going on elsewhere in the skeleton as far as structural parameters and cellular parameters are concerned. Iliac crest biopsies may not be representative, however, with respect to the amount of osteoid and the extension of osteoid seams.

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