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Iliac Cancellous Bone in Drug Addicts: A Histomorphometric Study

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ABSTRACT: Histomorphometry was used to determine structural bone changes in drug addicts. Iliac crest bone biopsies were obtained at autopsy from 28 subjects (21 male, 7 female, aged 18 to 45 years) who had a history of drug abuse and had died due to overdose of illicit drugs. For histomorphometry, undecalcified sections were investigated using the Merz grid. The following histomorphometric indices were measured and calculated: BV/TV, BS/BV, Tb.Th, Tb.N, Tb.Sp, OV/TV, OS/BS, Ob.S/BS, O.Th, ES/BS, Oc.S/BS, and N.Oc/T.A. In 28 controls (24 male, 4 female, aged 17 to 47 years) trabecular bone specimens were investigated in the same way. The parameters in drug addicts did not show any correlation to age, body weight, height or sex differences. Trabecular bone volume and trabecular thickness were slightly but not significantly increased (BV/TV: 23.37 ± 5.77% (mean, SD), controls 22.23 ± 5.08%, $p = 0.434$; Tb.Th: 172.67 ± 36.83 mcm, controls 169.73 ± 36.13 mcm, $p = 0.764$). Only the eroded surface was significantly different to the controls (ES/BS: 8.16 ± 2.04%, controls 6.96 ± 2.17%, $p = 0.038$). We conclude that the incidence of metabolic bone disease in drug addicts is low.

KEYWORDS: forensic science, forensic pathology, drug abuse, bone histomorphometry, cancellous bone, substance abuse

Trabecular bone is known as a metabolically alert tissue, and metabolic bone diseases can severely complicate hormonal or intestinal disorders (1). Also, nutritional factors, including alcohol consumption, strongly affect bone remodeling dynamics (2). On the other hand, there are a number of diseases which could be considered as complications of chronic drug abuse (3). With respect to general alterations, in particular the immunological and the hormonal system has to be mentioned (4–7). Since bone metabolism is closely related to the latter (8,9) it is conceivable that bone remodeling is altered in drug addicts. However, there are only a few investigations on this subject. The purpose of the present study is to detect possible changes of cancellous bone structure by means of bone histomorphometry.

Material and Methods

Iliac crest bone samples were obtained at autopsy from 28 subjects (21 male, 7 female, aged 18 to 45 years) whose death was associated with drug abuse. Data from these subjects are shown in Table 1. Information about drug consumption habits was very sparse. All subjects but one had scarred cubital veins, indicating a long period of intravenous drug abuse. One man without scarred veins was known to smoke heroin over many years. Postmortem toxicological findings as a rule revealed multiple drug abuse.

All bone specimens were fixed in Carnoy's solution and embedded in methylmethacrylate. Sections were cut and stained with the Goldner's trichrom method. Histomorphometric data were obtained by the point-counting method using a Zeiss microscope equipped with the Merz grid in one of the eye pieces. The site of measurement corresponded to iliac crest biopsy (10). For each biopsy, at a magnification of ×160, fifty grid fields were examined. Cortical bone and adjacent trabeculae were strictly excluded from measurement. The evaluated parameters (Table 2) were designated according to the nomenclature approved by the American Society for Bone and Mineral Research (11). Trabecular number and trabecular separation were calculated using the formulae given by Parfitt et al. (12). Iliac crest samples from 28 controls (24 male, 4 female, aged 17 to 47 years) who had died following acute injury with neither gross nor histologic evidence of bone-affecting illness were prepared and measured identically. The control sample was matched as closely as possible to the addict sample relating to the number of cases and age. The results were tested for distribution using the Lilliefors diagram. When data came from a Normal distribution the unpaired two-sample t test was performed to compare sample results with control values. If distributional assumptions were not met, the distribution free Mann-Whitney U test was used. For correlation testing the Pearson correlation coefficient was determined for the data that were normally distributed. Skewed data were tested using the Spearman rank correlation test. Using a two-level ANOVA (analysis of variance) the results were tested for sex differences (general linear models procedure from SAS). Because of the unbalanced sample the Type IV sums of squares were used.

In all tests a less than 5% probability of the difference having happened by chance was regarded as significant.

Results

With the exception of OS/BS, Ob.S/BS and N.Oc/T.A the data were normally distributed. The results of the histomorphometrical measurements are listed in Table 3. There was no relationship between the histomorphometric parameters and age, weight, or height. Sex differences were observed only in the control sample showing decrease of osteoid surface OS/BS (two-tailed P value 0.03564, see Fig. 1). Female values tended to be lower than male values in addicts too, but the difference was not statistically significant (two-tailed P value 0.06718). Interactions between group (gr = addicts and controls) and sex could not be verified (gr * sex: P value = 0.7328). The parameters of trabecular structure (BV/TV, BS/BV, Tb.Th, Tb.N, Tb.Sp) did not show any differences of statistical significance between drug addicts and controls. The raw data

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of bone volume and trabecular thickness (both with a slight but not significant increase) are plotted in Fig. 2. The parameters of appositional activity (OV/TV, OS/BS, Ob.S/BS, O.Th) did not show significant differences of the mean either. In the group of the resorptional parameters (ES/BS, Oc.S/BS, N.Oc/T.A), only the eroded surface (ES/BS) was significantly increased ($P = 0.038$; raw data are plotted in Fig. 2) compared to controls.

Discussion

To detect changes of bone mass Pedrazzoni et al. (13) investigated a group of 13 heroin abusers. The duration of abuse spanned a period of up to two years; the average daily intake was reported to be more than 0.5 g. With dual-photon absorptiometry the authors found a slight but significant reduction of lumbar bone mineral density. Another group of 14 former drug addicts with a clean period of 4 to 24 months did not reveal significant differences to the control group. The authors conclude that heroin abuse may alter bone metabolism. According to this Schnabel et al. (14) found histomorphometrically the mean thickness of trabeculae of the first lumbar vertebra in drug addicts to be lowered. Measurements of other histomorphometric indices are not reported in this paper.

The presented results of iliac crest bone measurements obviously do not support the findings mentioned. The histomorphometric data of our sample do not indicate alterations of bone metabolism and bone remodeling. There is only one parameter of bone resorption (ES/BS) slightly but significantly increased compared to controls. In particular there was neither any decrease nor increase of bone volume and trabecular thickness in the group of drug addicts.

The lower extend of osteoid surfaces in female controls compared to male controls is in accordance with the results of Melsen and Mosekilde (15), who reported on similar sexual differences of

TABLE 1—*Data of drug addicts and controls, given by mean, minimum, and maximum values.*

	Drug Addicts ($n = 28$)	Controls ($n = 28$)
Age	28.5 (18–45)	28.95 (17–47)
Weight, kg	74.64 (48–100)	78.65 (50–95)
Height, cm	177.25 (160–190)	178.65 (157–190)

TABLE 2—*Evaluated parameters, abbreviations and units.*

Parameter	Abbreviation	Unit
Cancellous bone volume per tissue volume	BV/TV	%
Trabecular surface per cancellous bone volume	BS/BV	mm^2/mm^3
Trabecular thickness	Tb.Th	mcm
Trabecular number	Tb.N	/mm
Trabecular separation	Tb.Sp	mcm
Osteoid volume per tissue volume	OV/TV	%
Osteoid surface per trabecular bone surface	OS/BS	%
Osteoid surface covered with osteoblasts (active osteoid) per trabecular bone surface	Ob.S/BS	%
Osteoid thickness	O.Th	mcm
Eroded surface per trabecular bone surface	ES/BS	%
Eroded surface covered with osteoclasts (active resorption surface) per trabecular bone surface	Oc.S/BS	%
Number of osteoclasts per tissue area	N.Oc/T.A	$/mm^2$

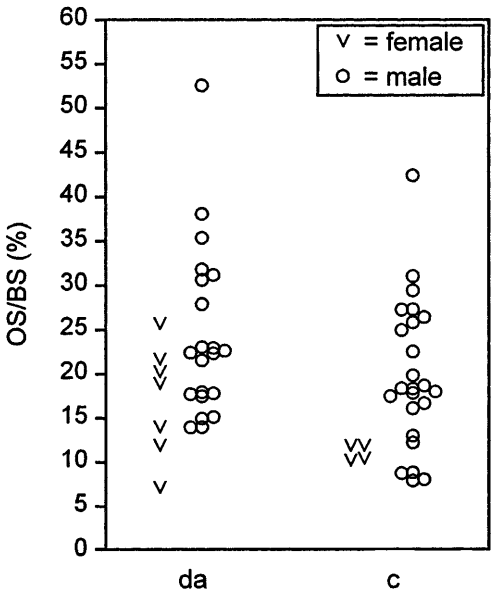

FIG. 1—*Plotted raw data of OS/BS. Only this parameter revealed a statistically significant sexual difference and only in the control sample:* da = *drug addicts,* c = *controls.*

TABLE 3—*Histomorphometric results of drug addicts and controls.*

	Drug Addicts ($n = 28$)			Controls ($n = 28$)			
	Mean	SD	CI_m	Mean	SD	CI_m	(P Value)
BV/TV (%)	23.37	5.77	21.13 to 25.61	22.23	5.08	20.26 to 24.20	0.434
BS/BV (mm^2/mm^3)	15.43	3.58	14.04 to 16.82	15.69	3.39	14.37 to 17.00	0.786
Tb.Th (mcm)	172.67	36.83	158.39 to 186.95	169.73	36.13	155.72 to 183.74	0.764
Tb.N (/mm)	1.34	0.15	1.28 to 1.40	1.31	0.17	1.25 to 1.38	0.516
Tb.Sp (mcm)	538.07	104.48	542.56 to 623.58	604.72	110.79	561.76 to 647.68	0.455
OV/TV (%)	0.71	0.32	0.59 to 0.83	0.68	0.38	0.53 to 0.82	0.673
OS/BS (%)	22.58	9.38	18.94 to 26.21	18.43	8.70	15.06 to 21.81	0.092*
Ob.S/BS (%)	2.87	1.93	2.12 to 3.62	2.85	2.89	1.73 to 3.97	0.976*
O.Th (mcm)	9.37	2.99	8.21 to 10.53	11.56	4.94	9.64 to 13.47	0.051
ES/BS (%)	8.16	2.04	7.37 to 8.95	6.96	2.17	6.13 to 7.80	0.038
Oc.S/BS (%)	0.62	0.37	0.48 to 0.76	0.83	0.49	0.64 to 1.02	0.084
N.Oc/T.A ($/mm^2$)	1.28	0.79	0.98 to 1.59	1.16	0.75	0.87 to 1.45	0.564*

Note: Values given by mean, standard deviation (SD), and 95% confidence interval of the mean (CI_m). The right-hand column shows the P values derived from unpaired t-test and Mann-Whitney U-test respectively, indicated by *.

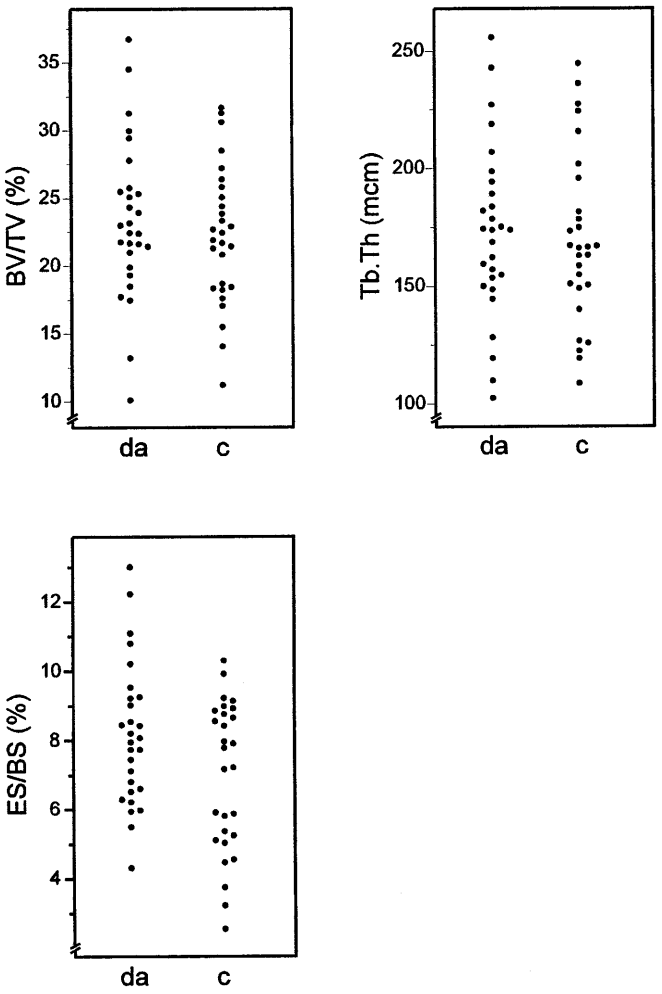

FIG. 2—*Plotted raw data of BV/TV, Tb.Th, and ES/BS:* da = *drug addicts,* c = *controls.*

this parameter. Furthermore, their investigations revealed lower extend of resorptive surfaces in the females, whereas we found this parameter not to be influenced by sex. But the samples might be too small to reveal reliable sexual differences.

Although drug abuse may interfere with bone metabolism, the incidence of metabolic bone disease in drug addicts seems to be low. But in forensic cases the information concerning the patients' history is often very sparse. In many of our cases we do not know the onset of addiction, facts about quantity and quality of the drugs abused, and frequency of abuse. Each of these facts can vary during the personal drug history and we know by experience that most of the addicts show very inconsistent consumption patterns. Therefore it is not possible to determine a homogenous group of ''drug addicts''. Despite scarred cubital veins and acute death associated with drug abuse our sample may include very differently advanced stages of drug dependent illness. The diagnostic value of bone histomorphometry, however, is most developed in cases with advanced structural changes (16).

The discrepant observations in lumbar vertebrae and iliac crest additionally may be a ''site error''. Though histomorphometry of the iliac crest bone is considered a valuable source of diagnostic information in metabolic bone diseases (17), it must be taken into account that histomorphometric results gained at one site of the skeleton are not representative of the skeleton at large. The intra-individual variability of bone volume may be substantial: Parisien et al. (18) reported differences between left and right iliac crest of about 10% and Dempster et al. (19) found the bone volume of the second lumbar vertebra 35% lower than that of the iliac crest. Krempien et al. (20) have shown marked differences of the histomorphometric parameters in the iliac crest, lumbar vertebra, femoral head, and distal femur. Furthermore, findings of the latter (20) and Malpe et al. (21) indicate that bone metabolism may vary at different skeletal sites. So it is conceivable that one skeletal site may reflect disturbances of bone metabolism earlier than another site. Pedrazzoni et al. (13) consider this different reaction to explain that, in their study, only lumbar BMD and not total body bone mineral (TBBM), measured by dual-photon absorptiometry, was lower in drug addicts.

In addition to metabolic bone disease, drug addiction can be complicated by bone infections (22–24). There are several reports on the effect of opioids on immune cells and infectious complications in intravenous drug addicts (25–27).

Furthermore, cases of painful diffuse osteosclerosis in hepatitis C positive intravenous drug abusers are reported recently: Beyer et al. (28) described a 28-year-old woman with radiographically increased density of almost all bones of the skeleton whereas the transiliac bone biopsy did not show changes of the bone mass. Villareal et al. (29) presented two cases with generalized increased skeletal density. One of the patients revealed mild radiographic osteosclerosis of the spine, long bones, and pelvis with the bone volume of the transiliac bone biopsy histomorphometrically within normal ranges. The other patient showed radiographically and histomorphometrically severe osteosclerosis. In both patients the osteoid volume was increased excessively. Whyte et al. (30) reported on another former drug abuser with diffuse sclerosis of almost all bones, sparing only the cranium. The reason for this increase of bone mass is still unclear. A viral origin of the disease seems to be more likely than stimulation of osteoblastic function by contaminant chemical substances or the drug of abuse itself (29, 31, 32). But as stated by Khosla et al. (33) ''the large variety of legal and illicit drugs taken by these patients will make it very difficult to identify the responsible agent''.

In conclusion, there are differing findings on structural changes of trabecular bone in drug addicts and further observations are necessary. For the possibility of both osteopenic metabolic bone disease and the syndrome of diffuse osteosclerosis in drug addicts, the incidence of both seems to be low, not only bone biopsies of the iliac crest and lumbar vertebra but also X-ray investigation of at least the axial skeleton and the long bones are necessary at autopsy in cases of death associated with drug abuse.

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