Differential patterns of altered bone formation in different bone compartments in established osteoporosis

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Abstract

Aim—To investigate the level of bone formation in the different bone compartments in cases of established osteoporosis, as previous work has concentrated on trabecular bone alone.

Methods—Bone formation rates were measured histomorphometrically, in the periosteal (P), cortical (C), subcortical (SC), and trabecular (T) compartments in iliac crest biopsies from 159 patients with established osteoporosis. The values were standardised using age and sex matched control data and patterns of differential change determined by analysis of parametric status (increased, normal, reduced).

Results—Mean bone formation was reduced in all four compartments. This was more marked (4.4/4.1 standard deviations below the mean in C/T, v 2.3/0.9 in P/SC) and more frequent (reduced in 81.5%/78.3% in T/C, v 43.3%/44% in P/SC) in the trabecular and cortical compartments

than in the periosteal or subcortical bone. Parametric status was equal in trabecular and cortical bone in 85.4% of cases, and in periosteal and subcortical bone in 65.7%, but in all four compartments in only 35.1%, indicating differential alteration of bone formation in the two sets of compartments (T/C v P/SC).

Conclusions—Altered trabecular bone formation is important in osteoporosis, but there are differential patterns of alteration in the other three compartments, emphasising the presence of different microenvironments in bone; thus the effect on the cortical compartment was similar to that on the trabecular, while the subcortical and periosteal compartments also showed linkage. The linkage between the two pairs was divergent, indicating different control of bone formation, with resultant different patterns of perturbation in osteoporosis.

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Keywords: osteoblast; osteoporosis; bone formation; bone compartments

Figure 1 Iliac crest biopsy showing location of compartments in which bone formation rate was measured (C, cortical; T, trabecular; P, periosteal; SC, subcortical).

Table 1 Mean values for both raw data and normal population values for bone formation in each compartment, together with spread of normal means and standard deviations

	Trabecular	Cortical	Periosteal	Subcortical
Mean normal value	0.59	0.72	1.9	3.7
Mean raw value	0.20	0.27	1.25	2.68
Spread of normal mean	0.4-0.69	0.6-0.79	NA	NA
Spread of normal SD	0.05-0.17	0.05-0.215	0.75	0.45

NA, not assessed.

Osteoporosis is a clinical syndrome characterised by a reduction in bone mass (osteopenia), and resultant low trauma fractures.¹ About 20% of human bone is trabecular and 80% cortical, though the trabecular bone is metabolically more active; osteoporotic fractures tend to occur at sites composed of more than 50% trabecular bone, such as the lumbar vertebrae, and the femoral neck.² As a result, most studies have concentrated on the reduction in trabecular bone,³⁻⁵ with little attention being given to the cortical,⁶ subcortical, or periosteal compartments, though subcortical bone thinning is known to cause decreased cortical bone width⁷ and reduced bone strength. Pharmacological treatment for established osteoporosis has largely been aimed at reducing osteoclastic activity,⁸ but unfortunately standard regimens are often unsuccessful in restoring bone strength and reducing fractures. There is evidence that reduced osteoblastic activity is an important factor in up to 80% of cases,⁹ and in a subgroup of these the reduction in bone formation is caused by failure of differentiation of osteoblasts,¹⁰ but these studies were limited to the trabecular compartment.

The methods available for monitoring the response to treatment provide a picture of the bone as a whole and are not specific to compartments. Monitoring the response to

Table 2 Mean z scores for bone formation in each compartment

	Trabecular	Cortical	Periosteal	Subcortical
Mean z score	-4.10	-4.43	-0.87	-2.28
SD of z scores	3.19	3.66	1.79	3.87

treatment by measuring bone density requires care to avoid the assumptions that bone density is a volumetric density, that treatment causes linear increases in bone mass, and that the pattern of bone loss is reversible.¹¹ All these assumptions will be altered if there is differential involvement in different compartments. Furthermore there is considerable debate regarding the value of different biochemical

indices for evaluating response to treatment,¹² and there is often discrepancy between changes in biochemical, densitometric, and clinical indices. In view of these discrepancies, and to determine whether there are contributions from the other compartments that might provide a more complete picture of the changes in bone mass and strength, we investigated the patterns of altered bone formation in each of the bone compartments in patients with osteoporosis.

Methods

Histomorphometric data from iliac crest biopsies were abstracted from the files of 159 patients referred to Hope Hospital, Salford, or the Manchester Royal Infirmary, Manchester, with established osteoporosis. The diagnosis of established osteoporosis was determined radiologically in each case by the presence of at least one non-traumatic vertebral crush fracture or a lumbar spine (L2–L4) bone mineral density less than two standard deviations below peak bone mass (assessed by dual x ray absorptiometry). The biopsies were fixed in absolute alcohol and processed three times in LR white resin monomer (London Resin Co), the last two processings taking place under reduced pressure. Resin polymerisation was carried out overnight at 60°C. Twenty seven 5 µm step serial sections were cut through each block with a Tungsten tipped knife on an LKB powered microtome. Groups of three sections were stained with toluidine blue (pH 4.2), or using the modified Giemsa or Von Kossa techniques. For fluorescence microscopy, 20 µm unstained sections were cut at different levels throughout the block. Histomorphometric analysis was carried out manually by an independent observer unconnected with the subsequent analysis. We only included biopsies in which there was an adequate core of bone, including both trabecular and cortical bone. Each patient had received two doses of oral dimethyl-chlor-tetracycline (10–15 mg/kg body weight), the first 15–18 days before the biopsy and the second 10 days later. Biopsy was performed within four to seven days of the last tetracycline dose.

When tetracycline labelled sections were analysed, the total length of mineralising surface (tLS) was divided into two components—those with two labels (double labelled surface, dLS) and those with only one label (single labelled surface, sLS). The former represented regions of the bone surface in which there had been mineralisation at the time of both of the two doses of label. Osteoid surface (OS) was identified in toluidine blue stained sections and defined as an unmineralised surface at least 3 µm thick. Indices were measured using standard techniques^{13–15} and defined according to the terminology proposed by Parfitt *et al.*¹⁶ Bone formation was measured in each of the four main compartments (fig 1). Trabecular and cortical bone formation rates were assessed by measurement of the trabecular and cortical apposition rates, which were calculated from the distance between the labelled surfaces divided by the time interval between the two label doses. The periosteal

Figure 2 Graph of raw value (black diamond) and corresponding age/sex matched mean (shaded square) of bone formation for each patient in each compartment: (A) trabecular; (B) cortical; (C) periosteal; (D) subcortical.

Figure 3 Scatterplots of z scores of bone formation in: (A) trabecular v cortical compartments; (B) trabecular v periosteal compartments; (C) trabecular v subcortical compartments; (D) cortical v periosteal compartments; (E) cortical v subcortical compartments; and (F) periosteal v subcortical compartments.

and subcortical bone formation rates were similarly assessed by measurement of the distance between the labelled surfaces. Each of the four variables (periosteal, cortical, subcortical, and trabecular bone apposition rates) were measured in each patient.

Results

Fully informative biopsies were available for 157 of the 159 cases initially selected (113 female, 44 male). The patients ranged in age from 26 to 75 years, with a mean (SD) age of 54.9 (11.3) years. The four variables in each patient were expressed as raw values and z scores. The mean and the spread of the raw values for each of the four variables are shown in table 1, together with the mean and spread of the population means against which the raw values were compared to obtain z scores. The population mean and standard deviation varied with age and sex for the trabecular and cortical apposition rates and so, while the appropriate age/sex matched means were used to produce the z scores for each variable in each patient, the mean and spread of the means is shown to give an overall impression of the deviation of the raw data from the norm. Similarly, the spread of the standard deviations of the normal values for each variable are also given in table 1. The population mean and standard deviation

were calculated across all ages for the periosteal and subcortical apposition rates.

The results showed a reduction in the mean raw value compared with the mean normal population value for each variable (table 1). The mean reduction was greatest in the cortical and trabecular apposition rates (4.4 and 4.1 SD below the mean, respectively), just over 2 SD below the mean for the subcortical apposition rate (2.3), and less than 1 SD below the mean periosteal formation (0.9) (table 2). The raw values and the corresponding age/sex matched normal population means, which therefore vary from patient to patient, for the entire data set of each variable are shown in fig 2.

To analyse the nature of these changes further, z scores were calculated for each variable in each patient to allow comparisons to be made between values from patients of differing age and sex (table 2). A z score for an individual value is the number of standard deviations by which the value differs from the mean of normal age and sex matched controls for the local population; z scores allow the standardisation of data and a z score of greater than either 2 or -2 was taken as abnormal. On this basis, the trabecular apposition rate was normal in 26 cases (16.5%), increased in three (2.0%), and reduced in 128 (81.5%), while the cortical apposition rate was normal in 30 cases

Table 3 Correlation coefficients between *z* scores of bone formation rates in each compartment

	Trabecular	Cortical	Periosteal
Trabecular			
Cortical	0.302		
Periosteal	0.267	0.177	
Subcortical	0.283	0.231	0.588

Table 4 Matrix of parametrical changes based on *z* scores of bone formation rates in each compartment

NNNN	6	NHNN	1	NLNN	5
NNNH		NHHH		NLNN	
NNNL	3	NHNL	2	NLNL	
NNHN	1	NHHN		NLHN	
NNHH		NHHH		NLHH	
NNHL		NHHL		NLHL	
NNLN	3	NHLN		NLLN	2
NNLH		NHLH	1	NLLH	
NNLL	37	NHLL	3	NLLL	12
HNHN		HHNN	1	HLNN	1
HNNH		HHNH		HLNN	
HNNL		HHNL		HLNL	
HNHN		HHHN		HLHN	
HNNH		HHHH	1	HLHH	
HNHL		HHHL		HLHL	
HNLN	3	HHLN		HLLN	
HNLH		HHLH	1	HLHH	
HNLL	2	HHLL	1	HLLL	3
LNNN		LHNN		LLNN	3
LNNH		LHHN		LLNH	
LNLL	2	LHNL		LLNL	1
LNHN		LHHN		LLHN	
LNHH		LHHH	1	LLHH	
LNHL		LHHL		LLHL	
LNLN	2	LHLN		LLLN	2
LNLH		LHLH		LLLH	
LNLL	17	LHLL	1	LLLL	39

Each cell is given in the format: periosteal, subcortical, trabecular, and cortical compartments, each variable being designated either normal (N), high (H), or low (L). The number of cases with each combination of changes is indicated.

(19.1%), increased in four (2.6%), and reduced in 123 (78.3%). Conversely, the periosteal apposition rate was normal in 76 cases (48.4%), increased in 13 (8.3%), and reduced in just 68 (43.3%), while the subcortical apposition rate was normal in 77 cases (49%), increased in 11 (7%), and reduced in just 69 (44%).

The *z* scores for each variable were plotted against each other (fig 3). These scatter plots showed no clustering of patients, and, except between the periosteal and subcortical appositional rates, a poor correlation between the variables (table 3). However, in view of the clear difference in the proportion of cases showing reduction in either trabecular/cortical, or subcortical/periosteal apposition rates, we made an attempt to identify subgroups within the data set. Each of the three variables in each patient was designated on the basis of its *z* score as either high ($z > 2$), normal ($2 > z > -2$), or low ($z < -2$), as explained above, and a four dimensional matrix used to determine the number of patients presenting with any given combination of changes (table 4). Six groups of patients with similar changes were identified; only those combinations of changes represented in more than three cases

Table 5 Demographic details of patients in the different histomorphometric groups

	M:F (n)	Mean age (years)	Age range (SD)
Group 1	17:22	56.1	29 to 74 (11.6)
Group 2	2:35	56.2	26 to 75 (10.1)
Group 3	4:13	55.8	42 to 72 (8.0)
Group 4	3:9	52.2	32 to 69 (11.5)
Group 5	2:4	57.0	48 to 72 (8.9)
Group 6	2:3	44.0	27 to 53 (10.8)

Table 6 Mean *z* scores of histomorphometric variables in the different groups

	Trabecular	Cortical	Periosteal	Subcortical
Group 1	-6.28	-6.29	-2.45	-6.23
Group 2	-3.54	-5.09	-0.15	-0.18
Group 3	-4.94	-4.77	-2.36	-1.42
Group 4	-5.63	-5.10	-0.97	-5.18
Group 5	-1.06	-1.10	-0.12	-0.23
Group 6	-0.14	-0.34	-0.39	-5.23

were judged sufficiently frequent to be considered as groups. Demographic details for each group are given in table 5 and the mean *z* scores for each of these groups are shown in table 6.

Group 1 was the largest and contained 39 patients with a reduction in all four variables. Group 2 contained 37 patients with reduced cortical and trabecular formation, but normal periosteal and subcortical formation. Group 3 contained 17 patients with reduced formation in the periosteal, cortical, and trabecular compartments, and normal subcortical bone formation. Group 4 contained 12 patients with reduced formation in the subcortical, cortical, and trabecular compartments, and normal periosteal bone formation. Bone formation was reduced but within normal limits in group 5, comprising six patients, while the smallest group (five patients) showed reduced bone formation in the subcortical compartment only. The mean age was similar in all of the groups (52.2 to 57 years) except group 6 (44 years), and was also similar to the overall mean age (54.9 years).

The designation of change (none, increased, or reduced) was equal in the trabecular and cortical compartments in 134 cases (85.4%). Of these 134 cases, the designation of change in both the periosteal and subcortical compartments was the same as that in the trabecular and cortical compartments in 47 cases (35.1%), but different in either the periosteal or subcortical compartment in 87 cases (64.9%). Of these latter 87 cases, the change in the periosteal and subcortical compartments was the same in 41 cases (47.1%). Overall the designation of change was the same in the periosteal and subcortical compartments in 88 cases (65.7%).

Discussion

The results confirm the high frequency of reduced bone formation in the trabecular compartment in osteoporosis (81.5%), but also indicate a similarly high frequency of reduced bone formation in the cortical compartment (78.3%). The correlation between these two variables was low (0.3), though when the cases with a similar designation of change were

analysed according to their z score, the level of bone formation was equal in the two compartments in 85.4% of cases. Likewise, the distribution of the level of bone formation was similar in the periosteal and subcortical compartments, with low formation in 48.4% and 49%, and normal formation in 43.3% and 44%, respectively. Additionally, the designation of change in the periosteal and subcortical compartments was the same in 65.7% of cases.

It was of note that the designation of change differed between the periosteal/subcortical compartments and the cortical/trabecular compartments in 64.9% of the cases in which the level of formation in the latter was equal, and it was the same in just 35.1%. Furthermore, of the cases in which the level of formation differed between the two sets of compartments (cortical/trabecular, periosteal/subcortical), the designation of the level of bone formation was the same in the periosteal and subcortical compartments in nearly half (47.1%).

These results show grouping of the four compartments into two separate sets, cortical/trabecular and periosteal/subcortical, with different patterns of altered bone formation in osteoporosis. Bone formation was reduced more often in the former group than in the latter (78.3%/81.5% *v* 43.3%/44%). The degree of reduction was similar in the cortical and trabecular compartments (mean z scores of -4.43 and -4.1, respectively), though the degree of reduced bone formation was greater in the subcortical than the periosteal compartment (mean z scores of -2.28 and -0.87, respectively). This was reflected in the wider range of z scores for the subcortical compartment, as well as a lower minimum z score (-8.22 *v* -2.53). Conversely, both the range and maximum and minimum z scores were similar in the cortical and trabecular compartments. However, notwithstanding this difference in the degree of reduced bone formation between the periosteal and subcortical compartments, they show close linkage of the level of bone formation, while the correlation between their z scores was also higher than for any other combination (0.59). This indicates close linkage of the level of bone formation in the periosteal and subcortical compartments, which are often spared from the reduction in bone formation common in the cortical and trabecular compartments—between which there also appears to be linkage of bone formation. This suggests that fundamentally different processes are involved in the normal control—and consequent perturbation by osteoporosis—of bone formation in the two sets of compartments. The nature of such differences is speculative, but possible candidates from animal and human studies include differences in the response to hormones within different compartments,^{17, 18} and the different modulation of strain which occurs through structures as opposed to at their surfaces.¹⁹ Obviously, in order to reduce the effects of osteoporosis on bone strength it will be most important to concentrate efforts on augmenting cortical and trabecular bone formation, should different methods of treatment be shown to be more or

less specific for one or other of the compartments studied. It is interesting that the compartments contributing most to bone strength were also those most frequently affected.

No formal clusters were identified after initial study of the scatter plots, which showed that the data were normally distributed over a wide area. However, as we have previously shown, even when there are no discrete clusters in a data set there is still a clinical and categorical value in splitting or subdividing the data using set limits, such as the upper and lower limits of the normal distribution,²⁰ as in this case. Using z scores of -2 or +2 to divide the data set into low, normal, or high bone formation, and grouping those with a particular pattern of change in the four compartments, six groups were identified. The largest group showed reduced formation in all four compartments, though the number showing reduction in the trabecular and cortical compartments only was nearly as large. These two groups accounted for 24.8% and 23.6% of the cases, respectively, constituting the majority of cases of osteoporosis.

This underlines the observation that the periosteal and subcortical compartments are often affected differently from the trabecular and cortical compartments, but also indicates that there are large numbers of patients in whom the periosteal and subcortical compartments are normal. These patients may have less severely deranged biochemical indices and more impressive biochemical evidence of bone formation owing to the contribution made by these two compartments, but without a corresponding increase in overall bone density and mechanical strength because of a reduction in the size of the compartments. Additionally, they indicate the presence of a profound biological difference in the response of these compartments to the variety of disease factors responsible for osteoporosis, as well as further emphasising the clinical heterogeneity of the condition. That the distinction between the two groups is not artificial is shown by the large differences between the groups in the mean z scores for the periosteal and subcortical compartments (-2.45 *v* -0.15 and -6.23 *v* -0.18, respectively). The two groups had a similar age profile, but females were much more common in the latter group (reduction in the trabecular and cortical compartments), suggesting that sex hormones may be responsible, though more precise determination of the cause or causes will require further work.

Both of the next two groups showed reduced cortical and trabecular bone formation, with similar mean z scores for both compartments. Group 3 had reduced periosteal bone formation, while in group 4 subcortical formation was reduced. The reason for this difference between the groups is unclear, but it is unlikely to be an artefact in group 4 since the z scores for the periosteal and subcortical compartments in both groups lay far from the -2 cutoff (though the mean scores were closer to the cutoff in group 3, and the possibility of artefact cannot be excluded for this group). The low

mean z score for the subcortical compartment in group 4 is mirrored in group 6, in which there is reduced formation in the subcortical compartment alone. Furthermore the reduction in this compartment is of similar magnitude in the two groups. The number of cases with reduced subcortical and normal periosteal formation (17 in groups 4 and 6) is equal to that for the converse, and there is no evidence of a biological preference for alteration in either of these two compartments. Interestingly, group 6 was the youngest and the only one with more males than females, but the small numbers involved reduce the value of these particular observations. Group 5 showed normal bone formation in all four compartments; osteoporosis was diagnosed clinically in each of these, but though bone formation was reduced in all cases, the degree of reduction (between 1 to 2 SD below the mean) was insufficient for a designation of "low" using a z score of -2 as the cutoff.

CONCLUSIONS

We have analysed histomorphometric data from a large cohort of osteoporotic patients to determine the nature of changes in bone formation in the different bone compartments. The results show close linkage of the level of bone formation in the periosteal and subcortical compartments, which are often spared the reduction common in the cortical and trabecular compartments—between which there also appears to be linkage of bone formation. Our data indicate that different processes are likely to be involved in the normal control—and consequent perturbation by osteoporosis—of bone formation in the two sets of compartments. The nature of these differences remains speculative, but the results emphasise the importance defining more precisely the different microenvironments in bone. Such studies will not only further our understanding of normal bone for-

mation, but will also help to clarify the reasons for differential involvement by osteoporosis, with possible consequences for treatment.

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