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The dynamic of mineralization

To better understand the mechanisms of action of therapies that reduce the risk of fracture, increase bone mineral density or change the biochemical markers of remodeling, it is necessary not only to analyse their tissue and cellular effects on bone remodeling, bone mass and microarchitecture, but also to discuss their influence on bone mineralization, one of the major parameters reflecting bone quality [1].

Mineralization is known to vary over microscopic regions, with age of the bone structural units (BSUs), the recently deposited ones being much less calcified than the older ones (Figure 1). During bone remodeling, after bone resorption, bone formation is a multi-step process. Following its deposition, the new matrix begins to mineralize after about 5 to 10 days from the time of deposition and the linear rate of this primary mineralization can be measured directly in vivo using double tetracycline labeling. After full completion of the BSUs (osteons in cortical bone or cancellous packets) a secondary mineralization begins. This process consists of a slow and gradual maturation of the mineral component, including an increase in the number of crystals and/or an augmentation of crystal size toward their maximum dimensions and/or an increase of the perfection at crystal level [2 –4]. The secondary mineralization progressively augments the mineral content of bone matrix deposited during primary mineralization, the latter representing only about 50 % of the maximum degree of mineralization (DMB) obtained at the end of the secondary mineralization phase [3 , 5 , 6]. Recent studies [7] in a ewe model – whose bone remodeling rate is similar to that in Humans – show that secondary mineralization lasts approximately for 24 to 30 months. This could mean that beyond 3 years of use of an anti-osteoporotic treatment the secondary mineralization does not increase.

Bone mineralization influences the mechanical strength of bone tissue [8 , 9] and its contribution to bone microhardness is well known [4]. The heterogeneity index of mineralization also influences bone strength. A homogenization of the mineralization makes the bone tissue more brittle [5].

Bone mineralization and microhardness in Humans: reference values

Contact microradiography of 100 µm-thick bone sections was performed [3 , 4] using a X-ray diffraction unit PW 1830/40 equipped with a diffraction tube PW 2273/20 (Philips, Limeil Brévannes, France). The nickel-filtered copper Kα radiation was used under 25 kV and 25 mA. The mineralization of bone is quantified using a microdensitometry computerized method and automated analysis of gray levels are used (MorphoExpert and Mineralization, ExploraNova, La Rochelle, France). A digital camera (pixel size: 2.83 µm at 2.5X) captures the microscopic image of the microradiograph, the values of the gray levels are obtained at the pixel level, and they are converted into DMB values expressed in g/cm^3 . Measured separately in cortical, cancellous, and total (cortical + cancellous) bone tissues, the main parameters describing the mineralization of bone are the DMB, the highest and most frequent DMB (DMB Freq. Max.) and the heterogeneity index (HI) of the distribution of DMB [3 , 4]. On the sections used for microradiography, microhardness (Hv in kg/mm^2) was also measured with a Vickers indenter and expressed as a mean of 60 measurements per bone sample (40 in cortical bone and 20 in cancellous bone) [4]. The mineral content of bone samples has also been evaluated by quantitative Backscattered Electron Imaging [10]. Carbon and aluminum were used for gray level references and osteoid and hydroxyapatite were employed as references to convert gray level values into calcium weight % values. A synchrotron radiation microtomography (SRµCT) method was also tested [5 , 6].

Parameters reflecting the mineralization of bone and its microhardness were measured in iliac bone samples from control men and women, and men and women with idiopathic osteoporosis (Table 1). DMB and microhardness values were significantly decreased ($p<0.003$) in osteoporotic patients versus controls. Heterogeneity index was not modified leading to a decrease of DMB without a modification in the distribution of mineralization. DMB and microhardness were not significantly different when measured separately in cortical and cancellous bone tissues. DMB and microhardness were lower in recent BSUs than in old interstitial tissue as quantified by focal measurements at the BSU level. There was a highly significant positive correlation between microhardness and DMB in control and osteoporotic patients ($r^2 = 0.36$, $p<0.0001$, Figure 2). Thus, the level of secondary mineralization appears to be the major cause of change in the microhardness of bone tissue, but mineralization explains only 40 to 50 % of the variance of the microhardness of bone tissue. These observations suggested that microhardness is influenced by other factors than mineralization, and organic matrix appears as a good candidate [4].

Effect of accelerated bone remodeling on mineralization

In adult bone, the major biological determinant of mineralization is the rate of turnover. Thus, an augmentation of the turnover induces a decrease of the "lifespan" of BSUs, i.e., of the time available for the secondary mineralization. This leads to the fact that new BSUs are eroded before they have fully completed their secondary mineralization, as proven by the presence of a large amount of BSUs that are not completely mineralized and a low mean DMB [3 –5].

Thus, in eleven cases of typical primary hyperparathyroidism (mean age 50±17 years) with hypercalcemia and renal calculi, the degree of mineralization of bone is significantly ($p<0.05$) lower (0.90±0.07 g/cm^3) than control patients of the same age (1.09 ± 0.08 g/cm^3). The heterogeneity index is not significantly modified (0.25±0.07 g/cm^3 and 0.25±0.08 g/cm^3 , respectively), which is in line with the shift towards low values of the distribution of mineralization. These results are similar to the ones reported in osteoporotic patients after treatment with teriparatide [11 , 12].

Effect of reduced bone remodeling on the degree of mineralization

A marked reduction in the "birthrate" of BMU following the use of antiresorptive agents such as bisphosphonates, estrogen, SERMs, prolongs the "lifespan" of the BSU, allowing a more complete secondary mineralization [13 –16]. This leads to an increase in the DMB with biomechanical effects (mainly an augmentation of the hardness). The variations of the remodeling activity not only affect the degree of mineralization but also the heterogeneity index of mineralization. In women treated with alendronate, the index is, after 2 years of treatment, similar to the one reported in premenopausal women. However, it is lower after 3 years of treatment. If such a trend was confirmed after long-term treatments (>5 years), this could be detrimental to the quality of bone tissue and its biomechanical properties.

Bone mineralization and strontium ranelate (Protelos®)

Strontium ranelate has a dual effect on bone remodeling by increasing bone formation and decreasing bone resorption, leading to prevention of bone loss and increase in bone mass and strength in rats [17]. Studies in monkeys [18] as well as observations in post-menopausal osteoporotic women treated for 3 years with strontium ranelate (2g/day) [19 –21], have allowed us to evaluate: 1) the relative calcium, phosphorus and Sr bone contents, 2) the distribution of Sr in cortical and cancellous bone, 3) the interactions between Sr and mineral at crystal level, 4) the influence of Sr on the DMB and, 5) the bone clearance of Sr over short periods of time after cessation of administration. In treated women, Sr is deposited in newly formed BSUs mineralized during the therapeutic period. Sr is adsorbed onto the mineral surface rather than substituting calcium ions. DMB is not different in women treated either with strontium ranelate or placebo groups. These data suggest that the increased BMD observed during strontium ranelate treatment could be due, in a major part, to an improvement of bone microarchitecture [22].

Conclusion

The degree of mineralization of bone is a determinant of its mechanical strength and hardness. It is influenced by the level of activity of bone remodeling. Quantitative studies of bone mass, trabecular microarchitecture, bone organic matrix and the degree of mineralization of bone are required to explain the anti-fracture effect of therapies at the tissue-level, and associated increases in lumbar bone density.

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Figure 1

Human bone samples. Left, microradiograph of a 100 ± 1 μm-thick section illustrating the heterogeneity of the mineralization in the various BSUs (young bone) and in the interstitial old bone tissue. The brighter interstitial lamellae had a mineral content of about 1.40 g/cm^3 while the least highly mineralized BSUs had a mineral content of about 1.00 g/cm^3 . Right, unstained section of endocortical bone with the Vickers impressions allowing to calculate the microhardness of bone tissue (about 45 kg/mm^2).

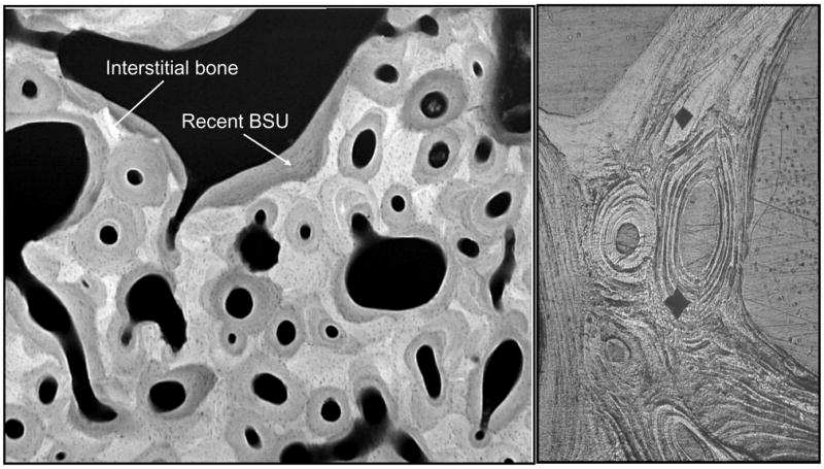

Figure 2

Measured separately in 421 BSUs from 21 control and osteoporotic patients, Vickers microhardness was significantly (r^2 =0.36, p<0.0001) correlated with the degree of mineralization of bone.

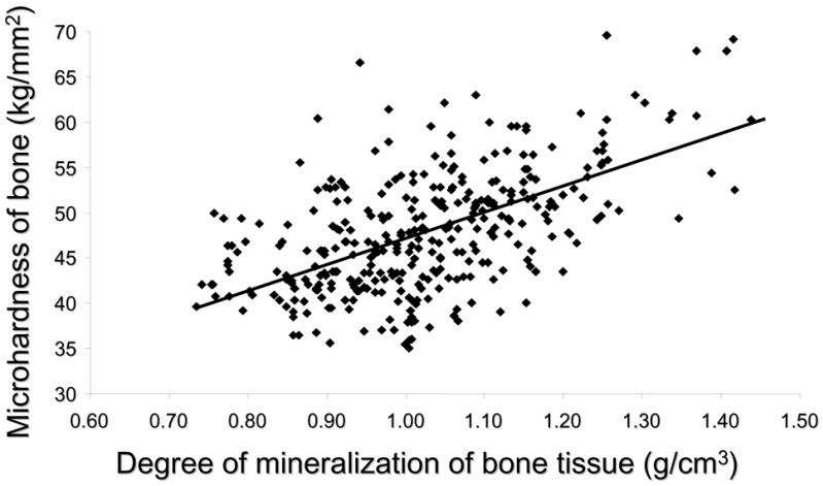

Table 1

Human control and osteoporotic (OP) patients. Mean values (±SD) for the Vickers microhardness (Hv). the degree of mineralization of bone (DMB) and the heterogeneity index of the distribution of DMB (HI).

Humans patients	Total bone tissue			Cortical bone tissue			Cancellous bone tissue		
	Hv_{25} (kg/mm^2)	DMB (g/cm^3)	HI (g/cm^3)	Hv_{25} (kg/mm^2)	DMB (g/cm^3)	HI (g/cm^3)	Hv_{25} (kg/mm^2)	DMB (g/cm^3)	HI (g/cm^3)
19 controls	49.18 ±1.82	1.10 ±0.09	0.22 ± 0.07	49.30 ±2.16	1.10 ± 0.09	0.21 ±0.07	48.92 ±1.57	1.11 ±0.08	0.19 ±0.05
52 OP	44.10 ±4.72*	1.03 ±0.07*	0.25 ± 0.07	44.05 ±4.69*	1.01 ±0.07*	0.24 ± 0.06	44.23 ± 5.07*	1.07 ±0.08*	0.23 ± 0.06*

* $p < 0.001$ versus controls