Calcif Tissue Int (1986) 38:323–327

Calcified Tissue International

Bone Remodeling During the Development of Osteoporosis in Paraplegia

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Summary. Osteoporosis developing during the first weeks after the onset of traumatic paraplegia was studied with cortical and cancellous samples of iliac crest and tibia of 14 patients, and compared to normals. We used a procedure of bone particle fractionation (according to degree of mineralization) that allowed us to establish a profile reflecting the metabolic remodeling of bone and to analyze the organic matrix of the newly synthesized tissue. In paraplegics, we observed a large increase in the proportion of little calcified bone in the cortical as well as in the cancellous bone. Based on amino acid analyses, we found a decreased number of hydroxyproline residues in the newly synthesized organic matrix from paraplegia bone resulting either from an alteration of the prolyl hydroxylation or from the presence of an excess of noncollagen polypeptides. These results, together with previously published data reporting increased urinary hydroxylproline and calcium kinetic parameters, suggest an enhanced rate of skeletal remodeling in acute paraplegia. When investigated 2 years after injury, the patterns of distribution approach that of normal subjects.

Key Words: Osteoporosis — Paraplegia — Bone remodeling — Bone matrix — Bone mineralization.

All paraplegic patients develop, within the first few weeks after injury, osteoporosis in the bones located below the spinal cord lesion. This type of neurologic osteoporosis seems to depend on local metabolic change. Neurologic lesions, however, produce vascular disturbances which may also be important in the pathogenesis of this type of osteoporosis [1].

In paraplegia, this sublesional osteoporosis (occuring below the neurologic lesion) is so striking that it may be observed by conventional radiographic techniques as early as 6 weeks after the spinal cord injury. It has been reported, furthermore, that urinary hydroxyproline is largely increased in paraplegia, suggesting that bone remodeling is stimulated [2, 3].

To corroborate histomorphometric analysis [4], calcium kinetics, and scintigraphic studies—which enable one to detect skeletal metabolic hyperactivity in paraplegic sublesional osteoporosis [5]—we now report biochemical analysis of the bone collected by biopsy from paraplegic patients. The same were fractionated according to their density to establish a pattern of particle distribution reflecting the metabolic state of the bone [6]. These fractions were analyzed to search for potential alterations of the protein matrix of the bone. Both the cortical and spongy bone of the iliac crest and tibia displayed changes supporting an increased turnover of mineralized tissue and perhaps a defective posttranslational processing of collagen that could account for increased amount of collagen breakdown products in the urine.

Patients and Methods

Patients

Bone biopsy specimens (collected with an 8 mm diameter trocar) of the iliac crest and/or the tibia were performed in 14 traumatic paraplegics, 10 men and 4 women, 18–52 years old. Eleven samples were taken from the iliac crest and 10 from the tibia within the first 6 months following the spinal cord lesion. In four of these patients, a tibia biopsy was performed 2 years after the onset of the paraplegia. In parallel, we investigated a control group of 16 samples collected from the iliac crest, femur, or tibia of 10 men and 6 women, 23–55 years old. All of them had either

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suffered a violent death or had died after an acute affection causing death in less than 48 hours. Autopsy excluded the possibility of disease which could affect bone metabolism.

Fractionation of Bone Particles According to Density

The bone samples were completely cleaned of adhering tissues (muscles, tendons, ligaments, etc). The cortex and the medulla were separated, coarsely ground, and dehydrated by lyophilization. Delipidation was performed on lyophilized bone fragments by repeated washings with petroleum benzin. Bone fragments were reduced to fine particles by crushing them in a stainless steel mortar until the whole sample could pass through a 375 mesh sieve. The maximum size of the particles, 40 μm therefore represented a very small part of an osteone.

A continuous gradient of density (d), from 1.40 $g.cm^{-3}$–2.44 $g.cm^{-3}$, was performed in a centrifuge tube by mixing toluene-bromoform mixtures as described earlier. [8].

One hundred milligrams of bone powder were deposited through a sieve on the top of the organic gradient and covered with a 3 mm layer of distilled water. The tubes were centrifuged for 30 minutes at 10,000 RPM in a swinging buckets rotor SW 25 (Spinco) at 4°C. After centrifugation, the material floating at the junction between the layer of water and the organic solvent was collected (fraction 0 $d \leq 1.41$ $g.cm^{-3}$) and the particles suspended in the organic gradient were collected as aspiration from the top of the tube in four successive fractions presenting progressively increasing density. The mean density of each of these four fractions, measured by weighting a known volume of the liquid, was 1.65 $g.cm^{-3}$ (fraction 1), 1.92 $g.cm^{-3}$ (fraction 2), 2.08 $g.cm^{-3}$ (fraction 3), and 2.17 $g.cm^{-3}$ (fraction 4). The bone particles were recovered from the organic liquid by centrifugation after a large amount of alcohol-ether (95-5 v/v) was added. After two washings with the same solvent, the bone samples were dried overnight under vacuum in a dessicator and then weighed. The distribution of particles in each fraction was calculated as the percentage of total recovered bone particles. The recovery was 95.8 ± 3.2%.

Chemical Composition of the Bone Organic Matrix

Weighed aliquots of the fraction (0–4) of bone large enough for analysis were hydrolyzed for 24 hours at 108°C in redistilled 5.6 N HCL under a nitrogen atmosphere. The aminoacid analysis was performed in an automated aminoacid analyzer using a single column procedure. The amount of protein in the fraction was deduced from the aminoacid analysis. The proportion of collagen and noncollagen polypeptides (NCP) in the organic matrix was computed for each fraction assuming that collagen type I contains 100 residues of hydroxyproline per 1000, as described by Lapiere and Nusgens [6]. The results were expressed in mg of collagen and NCP per unit volume (cm^3). The calcium content of each fraction was determined using atomic absorption (Perkin-Elmer) and the phosphorus according to Eastoe [7].

Results

Fractionation of Bone Particles According to Degree of Mineralization

The distribution profiles of the particles fractioned according to density (fraction 0–4) from cancellous and cortical bone from tibia and iliac crest in normal subjects and paraplegics patients (within 6 months after the occurrence of the lesion) are illustrated in Figs. 1 and 2.

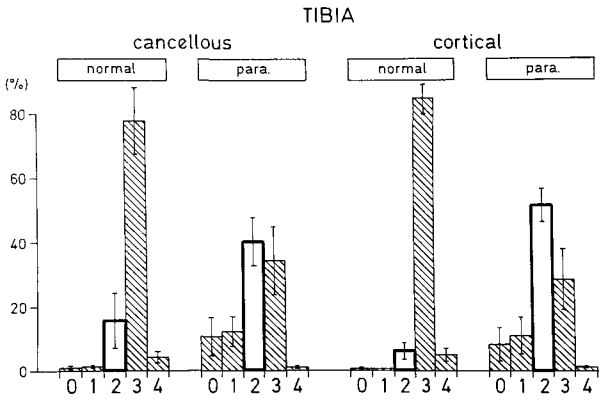

Fig. 1. Distribution profiles of bone particles (expressed for each fraction in % of total recovery) collected from the cortex (cortical) and spongiosa (cancellous) of tibia of 16 normal subjects and 11 paraplegic (para) patients, and fractionated according to their degree of calcification by isopycnic centrifugation in a continuous gradient of density. Fractions of increasing density are labeled 0 to 4 as detailed in Methods.

In cortical and cancellous bone collected from the tibia of control subjects (Fig. 1) 87% and 78% respectively of the particles are sedimented in fraction 3 (mean density: 2.08 $g.cm^{-3}$) and a few percents in fraction 2 (mean density: 1.92 $g.cm^{-3}$). There is no significant difference between the values observed in the youngest control as compared with the oldest, or between the males and the females (not illustrated). In bone from paraplegic patients, we observe a striking increase in the proportion of particles sedimenting in fraction 2 as well as in the least calcified fractions (0 and 1). Although the percentage of particles in fraction 2 is larger in the iliac bone as compared with the tibia in the normal subjects (Fig. 2), the increase of this fraction in paraplegic patients is highly significant in cortical as well as in cancellous bone ($P < 0.001$ by the Student's t test). When such a bone fractionation is performed on biopsy specimens from the tibia of paraplegics two years after the onset of the spiral cord lesion, the distribution profile of the bone particles is shifted towards that of the control subjects (Fig. 3), males being closer to normal values than females.

The calcium and phosphorus content was determined on each fraction from normal and paraplegic bones. They steadily increase from fraction 0 to fraction 3 and for a given fraction, display similar values in normal and paraplegic bone.

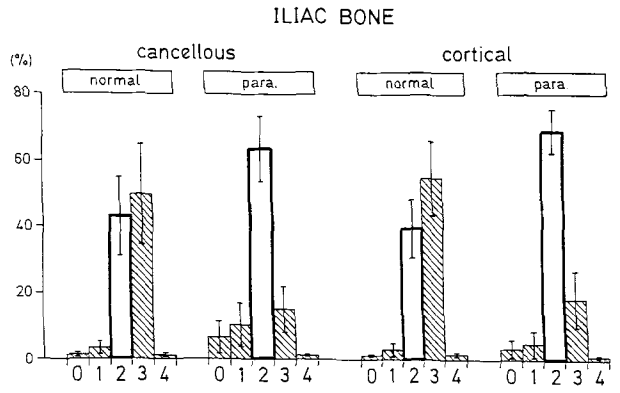

Fig. 2. Distribution profiles as described in Fig. 1 of bone particles collected from the cortical and cancellous of iliac crest of 11 normal and 11 paraplegic (para) patients.

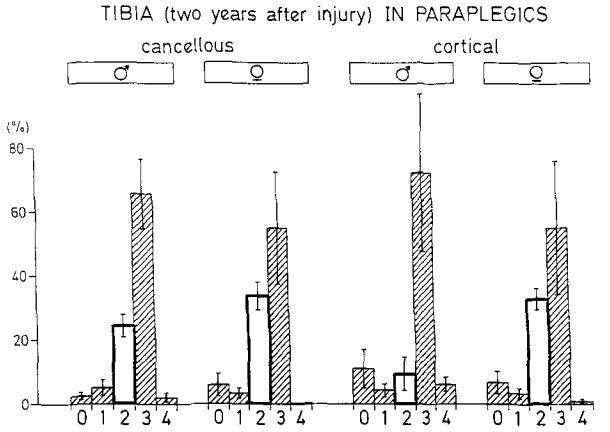

Fig. 3. Distribution profiles determined as detailed in Fig. 1 of bone particles collected from the cancellous and the cortical of tibia in 2 male and 2 female paraplegic patients 2 years after the spinal cord lesion.

Chemical Composition of Organic Matrix of Fractions

The amino acid composition of the organic matrix of the two largest fractions (2 and 3) in normal and paraplegic bone (iliac and tibia) were compared. The most representative amino acids are shown in Table 1. There exists a slight but reproducible difference (mainly in the least mineralized fraction 2 of iliac and tibia) in the proportion of hydroxyproline which is reduced. The hydroxylysine and the glycine content are not so significantly modified. In most samples of the iliac crest and tibia, proline is slightly higher in the paraplegic bone and leucine is also increased.

The concentration of collagen per unit volume of the fraction (in mg/cm^3) in the two largest fractions (2 and 3) is significantly lower whereas the amount of NCP is significantly higher in the bone of paraplegic patients as compared with that of the control subjects for the iliac bone. In the tibia, a similar difference was observed in fraction 2 but not in fraction 3.

Discussion

Regardless of its microarchitecture (osteone or lamellar), bone is composed of a mosaic of units presenting various degrees of mineralization. We have previously demonstrated that during the process of bone calcification, the progressive deposition of mineral salts onto an organic matrix made of collagen and NCP is irreversible and proceeds at a similar rate independently of the age of the subject [8]. The degree of mineralization of an osteone is related to the biologic age of the structure. The fractionation of bone particles (according to their charge in salts) allows us to obtain a profile of distribution which is therefore related to the metabolic activity of the tissue. A high rate of turnover, as during growth, will be associated with a high proportion of the least calcified fractions [6]. Adult normal human bone is composed of a large proportion of fully calcified matrix, as illustrated for the control subjects of this study. There exists, however, a difference between the distribution profile of the tibia and the iliac crest particles. In the latter, the proportion of fraction 2 is higher than in the tibia. This observation suggests that the iliac bone in normal subjects has a more active remodeling, which agrees with the conclusion of Meunier et al. [9]. Both sublesional bones (tibia and iliac crest), which were studied in our paraplegic patients, presented a distribution profile characterized by an increased proportion of little mineralized particles. This suggests that the sublesional bone in paraplegia is a newly formed tissue with a high rate of turnover, which may also be seen in other osteoporotic conditions [10, 11]. A marked increase in the urinary excretion of hydroxyproline [2, 3] and some parameters of 45 Ca kinetic studies [5] support this hypothesis.

The important osteoclastic bone destruction [4] observed at the beginning of the disease does not seem related to the endocrine status. Immunoreactive parathyroid hormone is, indeed, markedly reduced or even undetectable in paraplegic patients while serum calcitonin is increased during the first months of the paraplegia [12].

Furthermore, in our study, alkaline phosphatase levels were not statistically increased, whereas in other studies this parameter varies [2, 5, 13]. As reported, Minaire et al. [4] showed that the maximum resorption of the bone mass in paraplegia appears

Table 1. Amino acid composition of the organic matrix of the fraction 2 and 3 isolated from tibia and iliac crest bone of controls and paraplegics patients

	Fraction 2				Fraction 3			
	Norm. (7)[a]		Para. (6)[a]		Norm. (6)[a]		Para. (8)[a]	
	m	SEM	m	SEM	m	SEM	m	SEM
OH Proline[b]	89	± 2.8	78[d]	± 4.5	93	± 2.3	83[d]	± 4.1
Proline	112	± 5.3	117[c]	± 8	116	± 3	119	± 4.9
OH Lysine	3.5	± 0.4	2.9[c]	± 0.3	3.8	± 0.2	3.9	± 0.2
Lysine	32	± 1.4	35[c]	± 2.8	30	± 1.9	31	± 3.2
Glycine	302	± 9	283	± 10.3	315	± 4.8	311	± 14.5
Glutamic	80	± 1.6	79	± 2.5	77	± 1	78	± 3.1
Leucine	31	± 2.8	40[c]	± 3.3	29	± 0.6	31	± 2

[a] Number of controls (Norm) or paraplegic (Para) patients used for these analyses
[b] Only the most representative amino acids are quoted (in residues per 1000)
[c] $P < 0.01$; [d] $P < 0.05$

at the sixth month after the spinal cord injury. On the other hand, we demonstrated [3, 5] that in the first 3 months following the neurologic affection, there was an increase of the different parameters of Ca kinetics: Vo+, VT, and Vu. From the 2nd month, they progressively decrease to reach normal limits around the 6th or 7th month. This has been confirmed by other authors [13, 14]. From the latter study, it seems that osteoblastic function is not impaired by immobilization. Furthermore, there are two increases in active surface: an early one from the 4th to the 8th week, and the second one after the 16th to the 20th week. Thus, the chronology of events in spinal cord injury seems different from the sequence observed in normal subjects at rest. Such a study on normal subjects at rest failed to show the various modifications found in paraplegia [15, 16]. These observations may be explained by a difference in the intensity of the osteoporotic process in normal subjects during condition of rest and in paraplegic patients. Indeed, in paraplegics, demineralization below the neurologic lesion is sometimes demonstrable on conventional X-ray film within a few weeks after the trauma. Such a process is never observed in normal subjects at rest or even in astronauts after a long period of weightlessness. The biological consequence of this difference is obvious when one takes into account the highest level of hydroxyproline excretion which reached, in the paraplegics of our study, a value 3 times higher than in normal subjects at rest [5]. Thus, it appears that there is not only a chronological difference but also a quantitative difference between osteoporosis resulting from a spinal cord lesion and experimental disuse osteoporosis. These differences could explain the discrepancies in the assessment of endocrine functions of patients with "disuse" osteoporosis, since time and type of lesion are important factors.

The sublesional osteoporosis results most probably from a local metabolic alteration in which both synthesis and degradation of bone are largely increased. Even though the level of reconstruction is enhanced at the beginning of the affection, it cannot compensate for the loss of mineralized tissue. These periods of imbalance result in the development of osteoporosis. Such a high remodeling is required to explain the progressive enlargement of the bone diameter demonstrated radiologically [5]. In paraplegics investigated at least 2 years after the onset of the neurological lesion, the bone fractionation profile approaches that of our control subjects. After a very active period of bone remodeling, where degradation is more rapid than repair, there seems to be an evolution towards a new equilibrium between resorption and synthesis, although bone mass is at an "osteoporotic" steady-state the enlarged diaphysis keeps thiner than normal corticals.

The amino acid composition of the organic matrix of fraction 2 from normal bone demonstrates that it is composed of 90% collagen (calculated on the basis of hydroxyproline). In paraplegic bone, the observed decrease in the proportion of hydroxyproline residues might be the consequence of a dual process. It could imply a defect in the hydroxylation of proline, or an accumulation of NCP. In the former situation, it should result in an equal increase in the number of proline residues. Our results show an increase of only 5 proline residues instead of the expected 10, and a smaller-than-expected reduction of glycine. As the thermal stability of the triple helix of collagen is known to be affected by the degree of hydroxylation of the proline [17], a defective prolyl-hydroxylation could account for a higher susceptibility to degradation of the bone matrix in paraplegia. The presence of an excess of NCP in each fraction in paraplegic bone would explain the increase in some amino acids present in large proportion in glycoproteins (as leucine), and a smaller-than-expected increase in proline (much less abundant in NCP than in collagen).

Table 2. Concentration of collagen and NCP per unit volume of the fraction 2 and 3

Bone	Fraction 2 Subjects No.	Coll. m SEM	NCP m SEM No.	Fraction 3 Coll. m SEM	NCP m SEM
Iliac[a]	Para (3)[b]	515.5 ± 4.17[c,d]	127.8 ± 21.9[e] (7)[b]	471.9 ± 15.9[c,e]	122.1 ± 12.8[e]
(canc. + cort.)	Norm (6)	562.3 ± 21.4[d]	69.3 ± 6.7[e] (7)	534.4 ± 14.5[e]	40 ± 4.4[e]
Tibia[a]	Para (8)	439 ± 29.8[d]	163.8 ± 18.9[d] (6)	478.2 ± 30.7	81.8 ± 11.1
(canc. + cort.)	Norm (4)	491.8 ± 69.7[d]	95.5 ± 22.3[d] (4)	414.7 ± 40.8	74.8 ± 10.1

[a] Both cancellous and cortical bone were used for calculating the average
[b] Number of paraplegic and control subjects used for performing these analyses
[c] in mg/cm^3
[d] $P < 0.0125$; [e] $P < 0.001$

The calculated mean NCP is significantly higher in paraplegic patients than in normal subjects (Table 2). This calculation is, however, uncertain by the potential reduction of proline hydroxylation. A combination of the two processes might exist.

Conclusion

Our results demonstrate that the rapid osseous degradation observed at the beginning of the neurological lesion in paraplegia is immediately followed by an increase in active repair process. This important skeletal resorption is probably the most rapid in its evolution among the different pathologies of bone osteoporosis. These factors seem to differentiate the osteoporosis found in the various neurological affections associated with a paralysis, e.g., paraplegia from "disuse" osteoporosis. The term "immobilization" is misleading, since metabolic and bone disturbances are different in the various types of so-called disuse osteoporosis. Therefore, neurological factors could be a major contributory factor in the bone changes found in our patients.

Acknowledgments. We acknowledge the skillful assistance of Mrs. G. Rega for performing the density gradient fractionation of bone and of Miss J. Muhl for typing this manuscript. This research was supported in part by the Belgian Fonds de la Recherche Scientifique Médicale.

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Received March 12, 1985, and in revised form November 19, 1985