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Clinical Investigations

Bone Death in Hip Fracture in the Elderly

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Summary. We examined femoral head bone from 50 cadavers and from 21 patients who had suffered pathologic fracture of the femoral neck. We used a histochemical technique for lactate dehydrogenase (LDH) activity to demonstrate osteocyte viability. The femoral heads were removed within 36 hours of death or fracture, as LDH activity persists in the cytoplasm of viable cells for this time at 37° after interruption of the blood supply. In the controls, there was an age-related reduction in mean osteocyte viability, from 88 ± 7% (mean ± SD) at age 10–29 years to 58 ± 12% at age 70–89 years. In the hip fracture patients, mean osteocyte viability was 58 ± 21% but there was much variability in both osteocyte viability and bone mass. In 5 fracture patients, there was extensive osteocyte death, suggesting that most of the femoral head bone was nonviable; these patients had little microfracture callus. Others had predominantly viable bone which was usually osteoporotic, and their bone frequently showed microfracture callus. Osteomalacia was not seen in any patient. It is suggested that bone death, in addition to osteoporosis, may sometimes contribute to hip fracture in the elderly.

Key words: Femoral neck fracture — Osteocytes — Aged — Osteoporosis — Bone.

Hip fracture is an injury of major importance, occurring most frequently in elderly people; its mortality is about 10% [1]. Its victims suffer pain and prolonged hospitalization, and may subsequently be unable to maintain an independent existence. Hip fractures impose a heavy financial burden on the community; the acute care cost in the USA in 1986 was about 2 billion United States dollars [2].

An early histologic study of iliac crest bone from hip fracture patients concluded that the fracture was primarily a result of osteoporosis and osteomalacia [3]. More recently, a study of bone mass in the femoral neck, measured by dual photon absorptiometry, also found that hip fracture patients were more osteoporotic than were controls [4]. However, in a study of transiliac bone biopsies [5], we noted that, though hip fracture patients as a group were more osteoporotic than young controls, they were less so than were vertebral fracture patients, and that osteomalacia was rare. This lack of metabolic bone disease was confirmed by another Australian study [6].

We [5] also noted that the hip fracture patients were, as a group, considerably older than those presenting with vertebral fractures, and so considered the possibility that aging affected bone strength by a mechanism additional to that of osteoporosis. This has been shown to occur in vertebral bodies, though the mechanism was not identified [7]. One possible mechanism was bone death, and so, making the assumption that osteocyte death is synonymous with bone death [8], we established a method to demonstrate lactate dehydrogenase (LDH) activity in osteocytes [9]. The absence of LDH activity was considered to reflect osteocyte death, and its presence indicated that the cell had been viable at the time of hip fracture or death. In cadaver controls we noted that nearly all osteocytes were viable in young adults, and that osteocyte viability progressively decreased with age [10].

In the present study, we examined femoral head bone from patients with hip fractures to investigate the role of osteocyte death and other factors in the etiology of hip fractures.

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Materials and Methods

Subjects

We studied 21 patients (16 women, 5 men) who had suffered a subcapital fracture of the femoral neck. They were aged 82 ± 6 (mean ± SD) years, range 72–94 years. The fracture had occurred in each patient with little or no trauma. The femoral head was only accepted if it was removed within 36 hours of the fracture, as we have shown that LDH activity persists in osteocytes for 36–48 hours at 37° after removal from the blood supply [9]. After this interval, the fracture itself could lead to death of bone and bone marrow. The sample was also rejected if there was death of bone marrow, identified as a loss of LDH activity, or if viable osteocytes stained only faintly. These changes were always present in samples removed more than 3 days after a fracture, and were considered to indicate incorrect timing of the interval between the fracture and the removal of the femoral head.

The controls were 50 cadavers (16 women, 34 men) aged 11–89 years. Three were known to be chronic alcoholics but their full medical history was not available. All had died suddenly, and the femoral heads were removed within 24 hours of death. They have been previously reported in detail [10], but measurements of osteocyte viability were repeated by the same observer who studied the fracture sections (C. Dunstan) to avoid interobserver error. Bone area measurements were also repeated, as the measurement technique had been altered.

Methods

The femoral head was immersed in normal saline at 0–4°C immediately after removal and maintained at this temperature until processed (within 2 days). Coronal sections were cut through the entire face of the bone, at the level of the ligamentum teres, using a Microslice 2 precision saw (Metals Research Limited, Cambridge, England) fitted with a diamond sintered annular blade. The section included (1) two 200 μm thick sections for demonstration of LDH activity; (2) a 3 mm thick section from which an 8 × 10 mm block was cut for calcified bone histology; (3) a 2 mm thick slice for identification of microfractures.

Osteocyte Viability. Of the two sections used to demonstrate LDH activity, the one used to show osteocyte viability was rinsed in a jet of cold saline to remove most of the marrow, and was decalcified by immersion for 48 hours in 10% EDTA in 0.1 mmol/liter Tris buffer, pH 7.0 at 0–4°C. The second section was not decalcified and was used to show marrow viability. LDH activity in osteocytes and marrow was demonstrated by the tetrazolium-formazan reaction.[9] The sections were then fixed in buffered formalin, dehydrated in ethanol, and mounted in a xylene-based medium (Eukitt). Viable osteocytes were identified by the presence of blue formazan granules in the cytoplasm, and nonviable osteocytes were identified by their lacunar walls. For each section, at least 500 osteocytes were counted over 12 or more systematic random fields at an objective magnification factor of 40. Bone viability was defined as the percentage of lacunae containing viable osteocytes.

Calcified Bone Histology. The blocks of bone were fixed in formalin, dehydrated in ethanol, and embedded in a methylmethacrylate-glycolmethacrylate medium [11, 12]. Two 5 μm consecutive sections were cut on a Jung K microtome (A. G. Jung, Heidelberg). One section was stained with toluidine blue to show bone, osteoid, and cell detail, and the other was stained with alizarin red to demonstrate calcified bone. Bone area was measured on the alizarin stained section with a Kontron IBAS 2000 image analyzer. It was expressed as a percentage of section area.

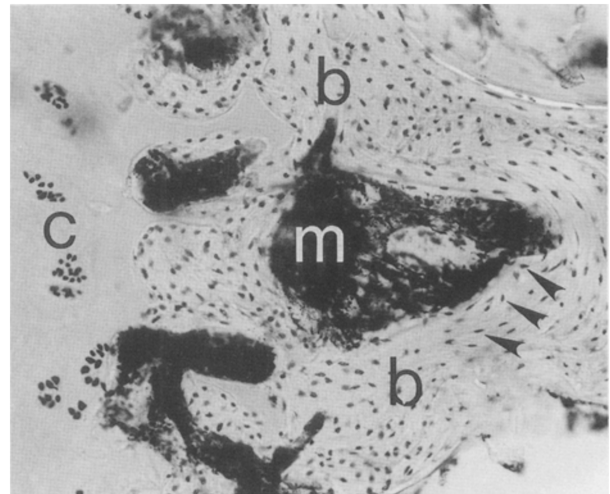

Fig. 1. Bone (b) from 20-year-old man, with nearly all lacunae containing viable osteocytes (small arrows) and with viable marrow (m) and cartilage (c). × 150.

Microfracture Callus. Marrow was removed from the bone by a pulsatile jet of water and the bone was allowed to air dry. A stereoscopic dissecting microscope with a magnification factor of 10 was used to identify and count areas of microfracture callus [10, 13].

Statistical Analysis

This was carried out using the "Minitab" statistics package (Pennsylvania State University). Linear regression was used to test the significance of correlations between variables.

Results

In the bone of control subjects, the osteocytes were nearly all viable at the age of 20 years (Fig. 1) whereas in older subjects there were scattered areas containing few or no viable osteocytes. In the fracture patients, there was more variation in bone viability, and in some there were large areas of bone with no viable osteocytes (Fig. 2).

Figure 3 shows the relationship between bone viability and age in both control subjects and hip frac-

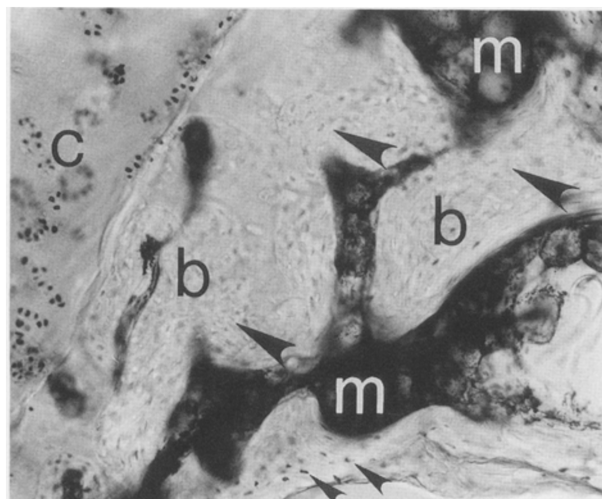

Fig. 2. Bone (b) from femoral fracture patient showing few lacunae containing viable osteocytes (small arrows) and large areas of bone with no viable osteocytes (large arrows), surrounded by viable marrow (m) and cartilage (c). × 150.

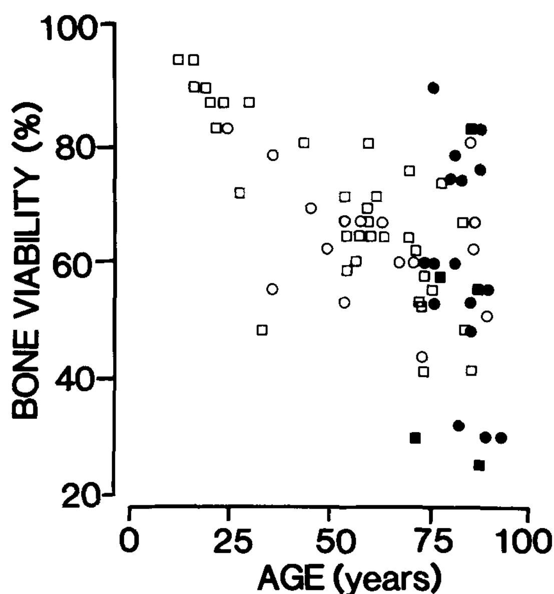

Fig. 3. Relationship between bone viability and age in controls and hip fracture patients. Male controls (□), female controls (○), male hip fracture patients (■), female hip fracture patients (●).

ture patients. In controls, bone viability was 88 ± 7% at age 10–29 years, and decreased to 58 ± 12% by age 70–90 years; there was a significant negative correlation with age (Table 1). The interobserver difference between C. Dunstan and S. Wong [10] was about 10%, so the values for controls are lower here than previously reported. In the fracture patients, the bone viability was 58 ± 21%. The values

Table 1. Relationships between measured variables

	Viability	Bone area	Microfracture callus
Controls			
Age	-0.68^{c}	-0.46^{c}	0.33^{a}
Viability		0.04	−0.24
Bone area			-0.28^{a}
Hip fracture patients			
Age	−0.18	−0.17	−0.20
Viability		0.12	0.63^{b}
Bone area			0.07

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$

were widely distributed and, in 5 fracture patients, the osteocyte viability was more than 2 standard deviations below the mean for the patient's age.

Figure 4 and Table 1 show the relationship between bone area and age in controls and hip fracture patients. In the controls, there was a significant negative correlation between age and bone area. Bone area in the hip fracture patients (17 ± 6%) was lower than in the 70 to 89-year-old controls (22 ± 6%) ($P < 0.01$). A valid measurement of bone area could not be made in 1 hip fracture patient with severe osteoporosis, as the bone sections contained many microfractures. The values for this patient were, therefore, excluded from Figures 5, 6, and 7.

Figure 5 shows the relationship between bone area and bone viability, expressed as mean ± SEM, and with the controls divided into four age groups. It shows the considerable reduction in viability by age 30–49 years, with the decrease in bone area being more marked in later decades. The low bone viability in the hip fracture patients and in the 70 to 89-year-old controls is also apparent. The heterogeneity of the data is clearer when individual values for bone area and viability from hip fracture patients and 70 to 89-year-old controls are examined (Fig. 6). It shows, in the hip fracture patients, the five very low values for viability and the six very low values for bone area, the latter all occurring in women.

In the hip fracture patients but not the controls, the number of areas of microfracture callus was positively correlated with bone viability (Fig. 7 and Table 1). Microfracture callus was uncommonly present when bone viability was very low. Microfracture callus was not significantly related to bone area in the hip fracture patients or in controls aged 70–89 or in controls aged 70–89 years (r = −0.08, NS).

Examination of the calcified embedded sections showed no evidence of osteomalacia in any patient or control.

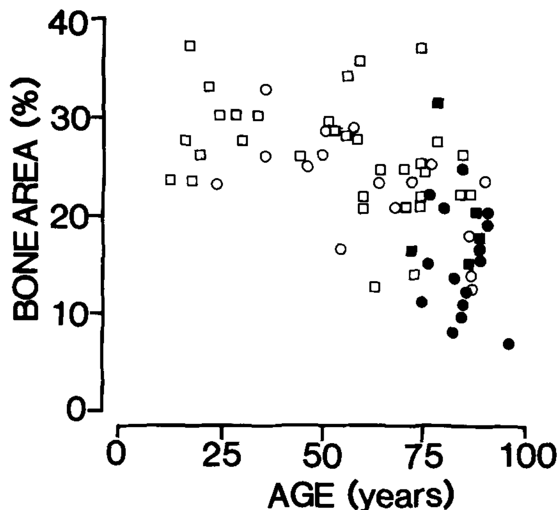

Fig. 4. Relationship between bone area and age in controls and hip fracture patients. The symbols are the same as in Figure 3.

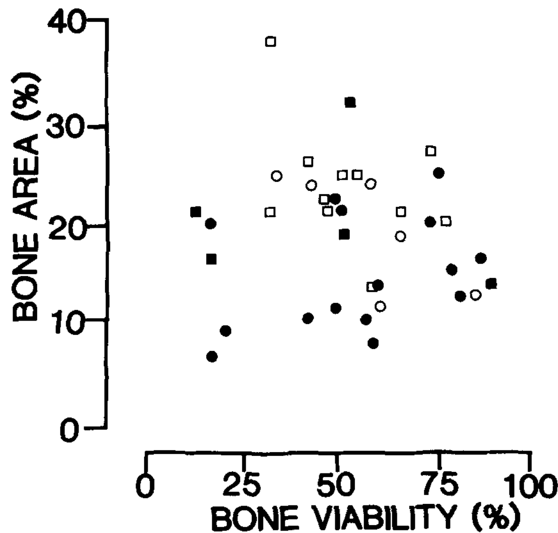

Fig. 6. Relationship between bone area and viability in fracture patients and controls aged 70–89 years. Individual data points are shown. The symbols are the same as in Figure 3.

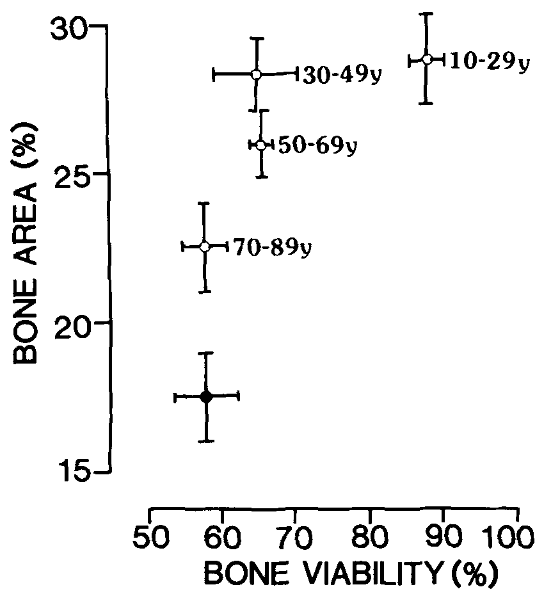

Fig. 5. Relationship between bone area and viability in controls of differing age groups (○) and in hip fracture patients (●). The data are shown as mean ± SEM.

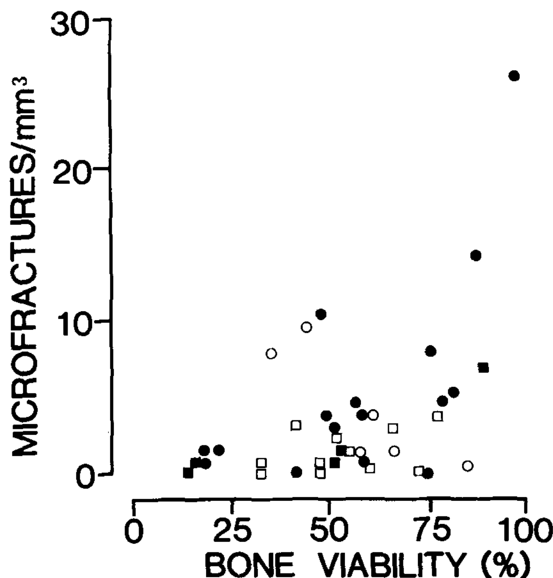

Fig. 7. Relationship between bone viability and microfractures in fracture patients and controls aged 70–89 years. The symbols are the same as in Figure 3.

Discussion

Bone viability in the femoral head decreased with age, and in some hip fracture patients there were large areas of bone containing no viable osteocytes. The precautions described in the methods section make it very unlikely that osteocyte death occurred as a result of the fracture. This interpretation is supported by the positive correlation between bone viability and microfracture callus. Bone area also decreased with age, and was lower in the fracture patients than in controls, probably reflecting the larger number of women in the hip fracture group. When the values for bone viability and area were related to each other, it was apparent that the lowest values for both viability and bone area were seen in the hip fracture patients though there was considerable overlap between the hip fracture patients and age-matched controls.

Osteoporosis is clearly a major cause of hip frac-

ture. Bone death also, when sufficiently extensive, may contribute in some patients by weakening the bone as it does in avascular necrosis of the femoral head [14]. The concept that bone death is sometimes one of the factors contributing to hip fracture is not negated by the similar mean viability of the patients and age-matched controls, as the pathologic findings are heterogeneous, with a wide range of values.

The cause of the osteocyte death is uncertain, but the findings in the controls indicate that age is one major factor. It could partly result from increasing age of the osteocytes, with failure to replace those that have died. This is consistent with the observation that, in both control and hip fracture subjects, dead osteocytes are frequently located in the deeper parts of the trabeculae. They are thus at the greatest distance from surface remodeling activity with its attendant renewal of osteocytes. Deep osteocyte renewal is dependent on the less active haversian remodeling systems. Vascular insufficiency, which affects many other regions with advancing age, is another possible cause of osteocyte death [15]. The femoral head is particularly susceptible to osteonecrosis, due largely to its vascular anatomy [14]. However, vascular injury to the femoral head characteristically produces osteonecrosis with distinctive reparative processes. The dead bone, containing no living osteocytes, is enveloped by new bone containing viable osteocytes. This pattern has been observed in rabbits with surgically induced osteonecrosis [16] and in the femoral heads of humans with osteonecrosis [17], with primary osteoarthritis [18] and with late osteonecrosis after hip fracture [19]. This pattern was not seen in any hip fracture patient in the present study. Therefore, the large reduction in viable osteocytes, whether due to diffuse ischemia, simple aging of cells, or some other pathologic process, does not result in the usual revascularization or repair.

Microfracture callus formation is another reparative process. In the present study, it occurred predominantly in living osteoporotic bone, and was uncommonly present when there was much osteocyte death. This callus formation is doubtless a response to recognized structural weakness and is perhaps not always associated with a microfracture. It presumably did not occur in the bone with much osteocyte death because, in the predominantly dead bone, the weakness was either not recognized, or the reparative process could not respond to it.

Osteocytes and their network of canaliculi may maintain the integrity of both the collagen matrix of bone, which provides its tensile strength, and of the bone mineral, which provides the compressive strength. Severe osteonecrosis of the femoral head frequently causes collapse of the bone [14], though the mechanism is not understood. It is not known whether the diffuse, moderate reduction in osteocyte viability in the fourth and fifth decades affects the strength of bone. However, there can be little doubt that, in some of the hip fracture patients, with a mean osteocyte viability below 25%, and with whole trabeculae devoid of viable osteocytes, bone strength would be reduced. The failure to repair areas of weakness by microfracture callus was also striking when many of the osteocytes were dead, and must also have contributed to weakness of the bone.

We have presented evidence suggesting that the osteocyte death occurring in the femoral heads of elderly people is not followed by the normal reparative processes and may contribute to hip fracture. Further studies are needed to relate bone viability to bone strength, and to determine the cause of the osteocyte death, so that prophylactic measures can be considered.

This study does not explain why hip fracture sometimes occurred when both the bone area and osteocyte viability were not low. No patient had osteomalacia. Cortical bone loss has been suggested as a cause of hip fracture, [20] but there is little cortical bone in the subcapital region where the fractures occurred in our patients. The associated fall would have contributed, but would not have been likely to cause a fracture in a young person. Finally, we cannot exclude structural deficiencies in trabecular orientation, or focal decreases in bone or viability not detected by our sampling procedures.

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