Bone Densitometry of the Femoral Midshaft in the Protein-Deprived Rat*

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SUMMARY

Densitometric assessment of radiographs of the femoral midshafts in protein-deprived and age-matched control rats, has shown a significant loss of total bone density in the protein-deprived group. This reduction is no greater than can be accounted for by the loss of cortical bone surface area, suggesting that while bone mass is reduced as a result of protein deprivation, the mineral composition of the residual bone is likely to be normal. These findings are supported by data on the ash content of extirpated bone in the same group of animals.

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The radiological diagnosis of osteoporosis is semi-quantitative, so that only gross changes are usually detected. Between 30% and 50% of bone mass must be lost before radiological reduction of bone density can be distinguished visually.[1,2] The need for a more sensitive method, permitting earlier detection of minor changes in mineral content and bone mass, has resulted in the introduction of quantitative radiological techniques, such as the direct measurement of cortical bone thickness.[3,4] However, significantly more information is yielded by densitometric scanning of bone radiographs.[5]

Our interest in the latter technique arose during the study of bone metabolism and composition on the protein-deficient rat. Rarefaction of bones has been described in experimental protein depletion,[6] but we were unable to distinguish by naked-eye examination of the radiographs any difference in bone density between protein-deprived rats and control animals. This article presents the results of examining the long bones, using a densitometric scanning device.

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METHODS

General Concepts

The technique employed was adapted from that of Albanese *et al.*[7] Conventional radiographs of the long bones of the rats were made. An aluminium step-wedge which was included with each radiograph (Fig. 1), minimized the possible error arising from variations in exposures and processing of the plates, thus serving as a reference standard.[8,9] That part of the radiograph displaying

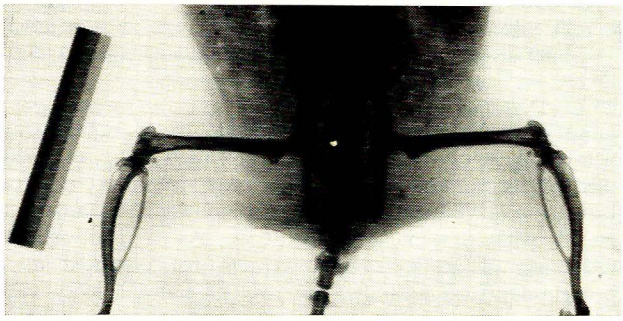

Fig. 1. Radiograph of hind legs of a rat, showing aluminium step-wedge used as an internal standard.

the hind limbs was then positioned on the scanning densitometer and a tracing made across the midshaft of both femurs of each rat, after appropriate adjustment of the sensitivity of the scanner, using the aluminium wedge as the standard.

The midshaft of the femur was studied since it is the most symmetrical and cylindrical part of that bone regarded as being representative of the total skeleton.[10] The tracings enabled a number of assessments to be made: (*i*) an accurate measurement of the outer width of the bone (B, Fig. 2); (*ii*) the medullary cavity width (B—2A, Fig. 2) measured as the interpeak distance, since the peaks correspond to the inner edges of the cortical bone;[11] (*iii*) the area under the graph, expressed as mm^2, using a Haff planimeter. This represented the 'total bone density' at the femoral midshaft.

Since the deflection of the densitometric recording needle is dependent on the optical density of the radiograph, the height of the deflection is a measure of both the mineral content of that bone and the actual amount of cortical bone. Thus the planimetric area obtained (total bone density), is influenced by both factors. In order to assess selectively the mineral content of the bone by this technique, the total bone density has to be evaluated in relationship to the quantity of bone, measured as the cortical surface area. This latter estimation was determined by measurement of the radii of the inner and outer circle of cortical bone (D and C, Fig. 2), calculating the circular areas subtended by the radii (πr^2), and subtracting the smaller from the larger area. Cortical thickness (A, Fig. 2, obtained by subtraction of radii alone) does not equate

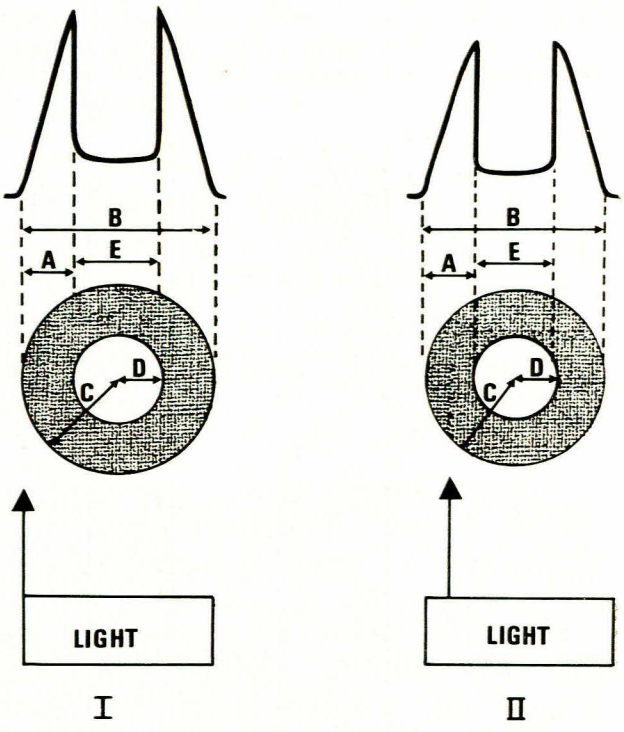

Fig. 2. Schematic representation of bone densitometry tracings of femoral midshaft from control (I) and protein-deficient (II) rats. The light source moves from left to right across the bone radiograph and the density is continuously recorded. A = cortical thickness, B = total bone width, C = the outer radius, and D = the inner radii. Cortical bone surface area is calculated by subtracting the circular area subtended by *D* from that subtended by *C*. The total area under the density curve is measured by planimetry and represents the 'total bone density'. Note that the bone density is less in the femoral midshaft of protein-deficient rats compared with that in the control animals in spite of nearly similar cortical bone thickness in both groups. The differences in the two groups can be accounted for by differences in the cortical surface areas (see Table I).

with total cortical surface area; the former can be identical in 2 rats with different surface areas, as shown in Fig. 2.

By dividing the total bone density by the cortical surface area, a factor *f* was obtained which expressed the relationship of these two factors. Any change in this relationship is likely to be due to a difference in contribution made by the bone mineral content.

Technical Aspects

1. Roentgenograms of the femurs of anaesthetized rats were simultaneously exposed with the aluminium step-wedge. The X-ray unit was a Philips Polytome operated at 35 kV, 19 Mas with 0,03 second exposure. The distance between the rat and the X-ray tube was 1,22 m. Cronex film was used; 90-second, automatic processing of the film was achieved with a Kodak Rapid Processor.

TABLE I. MEAN BONE DENSITOMETRIC MEASUREMENTS (± STANDARD ERROR) TAKEN AT THE FEMORAL MIDSHAFT OF PROTEIN-DEPRIVED AND AGE-MATCHED CONTROL RATS. THE PROTEIN-DEPRIVED GROUP SHOWED SIGNIFICANTLY LOWER PARAMETERS WITH THE EXCEPTION OF FACTOR f, SUGGESTING A PARALLEL REDUCTION OF TOTAL BONE DENSITY AND CORTICAL BONE SURFACE AREA

Rats	No. of measurements	Total bone density (planimetric area mm²)	2 x outer radius (mm)	2 x inner radius (mm)	Cortical bone surface area (mm²)	Planimetric area/cortical bone area (factor f)
I. Age-matched controls (20% casein)	**16**	**35,73 ± 2,83**	**2,99 ± 0,05**	**1,94 ± 0,04**	**4,11 ± 0,22**	**8,64 ± 0,41**
II. Protein-deprived (4% casein)	**16**	**23,60 ± 1,53**	**2,63 ± 0,03**	**1,72 ± 0,04**	**3,07 ± 0,09**	**7,68 ± 0,47**
Statistical comparison of I vs. II		**t = 3,763 $P<0,001$**	**t = 6,279 $P<0,001$**	**t = 3,577 $P<0,002$**	**t = 4,365 $P<0,001$**	**t = 1,536 $0,1<P<0,2$**

2. The wedge consisting of 5 steps with 0,5 mm increments was machined from aluminium alloy.

3. The densitometric apparatus consisted of a Hilgert and Watts H451 microdensitometer, with a Hilgert and Watts L454 motor. Scanning speed was 0,25 mm/min. The densitometer was coupled to a Hitachi 2 PD.54 recorder, the response being standardized to 1,5 millivolts.

Experimental Design

Protein depletion was produced in post-weanling rats as previously described by Stead.[12] Eight protein-deprived, and 8 normal age-matched controls were studied at the end of 5 weeks' feeding of 4% and 20% casein diets, respectively. Both femurs of each rat were examined radiologically as described. All measurements were done in duplicate.

RESULTS

These are shown in Table I, and schematically in Fig. 2. Total bone density, i.e. the total planimetric area under the bone density curve, is significantly reduced in the protein-deprived rats. Similarly, a significant reduction in outer and inner cortical radii is noted in the same group of animals, the diminution of the outer cortical radius being a little more marked. Cortical bone surface area, calculated from the cortical radii, shows a 25% drop in the protein-deprived rats; this accounts for almost all the reduction in the total bone density, as factor *f* is not signicantly different in either group of rats.

DISCUSSION

Standard radiographic techniques for the diagnosis of osteoporosis are semi-quantitative. Densitometric measurements with appropriate reference standards have, in recent years, allowed the accurate assessment of bone mineral density and cortical thickness.[1]

As part of our investigation into the bone metabolic consequences of protein deprivation, densitometric evaluation of bone radiology has enabled us to obtain quantitative data on bone density, combined with an indication of its mineral content. We have found a significant loss of total bone density in protein-deprived rats compared with age-matched controls. As a proportionate reduction of cortical bone surface area is noted in the protein-deprived animals, the loss of bone density seems to be a consequence of quantitative reduction of bone, rather than a change in its mineral content. This is supported by our unpublished studies on radio-calcium kinetics and bone ash content in the same group of protein-deprived rats, in which bone formation rate is reduced by about 50% without significant reduction in bone resorption, resulting in a reduced cortical bone mass. This is associated with a normal percentage ash content of the extirpated bone. Our findings of reduced bone mass with normal mineral composition, are in keeping with the observations of others in human[13] and experimental[8] protein deficiency. Our data also endorse the suggestion that radiographic measurements of cortical bone thickness may be of limited value when cortical porosis is suspected, as loss of bone substance may not necessarily be accompanied by alteration in cortical thickness.[14]

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