ACTA STEREOL 1994; 13/1: 55-60
PROC 6ECS PRAGUE, 1993
ORIGINAL SCIENTIFIC PAPER

DIRECT STEREOLOGICAL ESTIMATION OF 3-D CONNECTIVITY DENSITY IN HUMAN ILIAC CANCELLOUS BONE: THE EFFECT OF AGE AND SEX

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ABSTRACT

The use of a new, direct estimator of 3-D connectivity in arbitrary networks, the ConnEulor, based on the disector-principle is illustrated in a series of iliac crest bone necropsies. To obtain an unbiased estimate of connectivity or 'trabecular number' takes about 1 hour per specimen.

A rather pronounced relation between connectivity and age was seen in females, it corresponds to a halving of trabecular number over ~ 50 years. No such relation was observed in males. No correlation obtained between connectivity and trabecular volume density.

Key words: Age, Bone, Connectivity, Trabeculae, Sex, Stereology.

INTRODUCTION

Cancellous bone undergoes significant alterations with aging. In addition to reductions in bone mass, the microstructure of cancellous bone appears to be transformed from a highly connected set of trabecular plates to a more variably connected network of rods and trabecular elements (Parfitt et al., 1983). This apparent reduction in cancellous bone connectivity is particularly evident in women following the menopause (Becker et al., 1988). In both men and women, alterations in cancellous bone microstructure occur as a consequence of both normal and abnormal skeletal remodeling (Parfitt, 1984, Mosekilde, 1990). These changes contribute to the observed increase in skeletal fragility associated with aging (Mosekilde et al., 1987).

Quantitation of the topological changes in cancellous bone is critical in order to assess the importance and contribution of these changes to bone strength and ultimately to fracture risk. To date, most studies describing age-related changes in cancellous bone microstructure have been either qualitative (Mosekilde, 1990, Dempster et al., 1986) or limited to 2-D estimators of structure (Parfitt et al., 1983, Compston et al., 1987, Mellish et al., 1991).

With the development of the new stereological tools, the disector (Sterio, 1984) and the ConnEulor (Gundersen et al., 1993), it is now possible directly to estimate connectivity in biological specimens in a practical setting. To this end, we chose to determine the 3-D connectivity density of human iliac cancellous bone in males and females utilizing these new stereological tools and compared these findings with changes in cancellous bone volume.

MATERIALS AND METHODS

Transcortical iliac core specimens of a width of 7 mm were obtained from males (n = 18; age 21 to 84 years) and females (n = 16; ages 20 to 96 years) at autopsy. Necropsies were fixed in 10% neutral buffered formalin and processed undecalcified in methylmethacrylate (Schenk et al., 1984). Because the specimens were cylinders, they were embedded with random rotation with respect to the fixed, central axis of the biopsy. Twelve to 15 pairs of 10 μm vertical sections, separated by 20 μm, were prepared from each necroopsy in order to construct 30 μm physical disectors. To conserve necropsy tissue, the sections used in the present study were collected consecutively from the center of the necropsy and stained with a modified von Kossa stain (Schenk et al., 1984).

Section pairs were projected onto a tabletop at a final magnification of 16X utilizing two Olympus BH-2 microscopes (Olympus Corp. Lake Success, NY) equipped with projection attachments (Gundersen et al., 1988). Connectivity density was estimated using the ConnEulor counting principle (Gundersen et al., 1993). Briefly, the appearance of "holes", "islands", and "bridges" were enumerated in both directions (Figure 1).

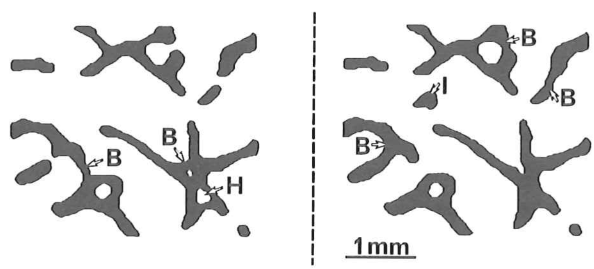

Fig. 1. Physical disector illustrating topological events in iliac cancellous bone as visualized in a section pair separated by 20μm. The appearances on either side of "holes" (H), "islands" (I), and "bridges" (B) are indicated in the figure.

Tissue volume and cancellous bone volume were estimated by point counting. All counts were limited to cancellous bone and associated marrow excluding endocortical surfaces.

Connectivity density was calculated as:

$$W_V = \frac{1}{2 \cdot h \cdot a(p)} \cdot \frac{\sum(B-H-I)}{\sum P} \quad (1)$$

where H, I, and B are the "holes", "islands", and "bridges", respectively. P is points hitting tissue, h is the disector height (30 μm), and a(p) is the area per point in the test system, corrected for magnification.

On average about 125 events (B+H+I) were counted per necropsy providing an expected $CE(W_V)$ of 0.13 (10). The "bridges" are clearly donimating in disectors through the trabecular network, B/(B+H+I) is 0.85, a factor of critical importance for counting efficiency (Gundersen et al., 1988). The counting takes about 1 hour per necropsy.

RESULTS

In females, significant age-dependent reduction in connectivity density was observed in iliac cancellous bone (Figure 3). The rate of loss of connectivity density corresponded to an approximate 2 fold reduction from about ages 30 to 80. A slight but non-significant reduction in cancellous bone connectivity density was also observed in males (Figure 2).

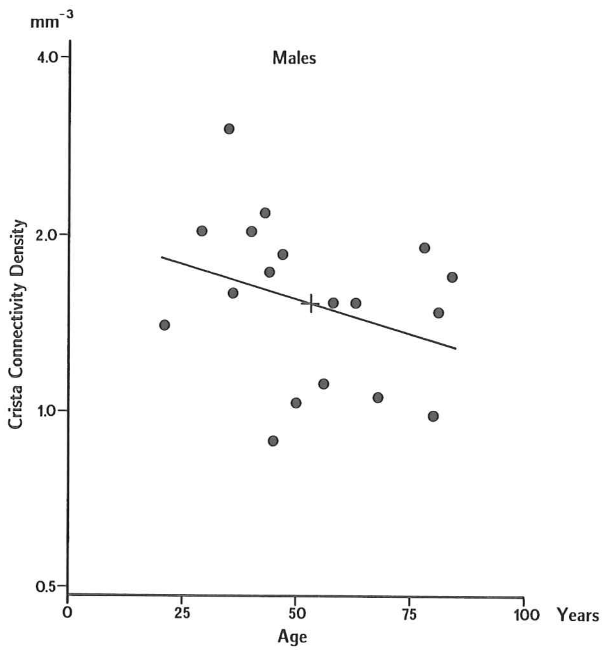

Fig. 2. Trabecular connectivity density, mm^{-3}, in iliac crest on a log ordinate as a function of age in males. The (non-significant) regression line is shown.

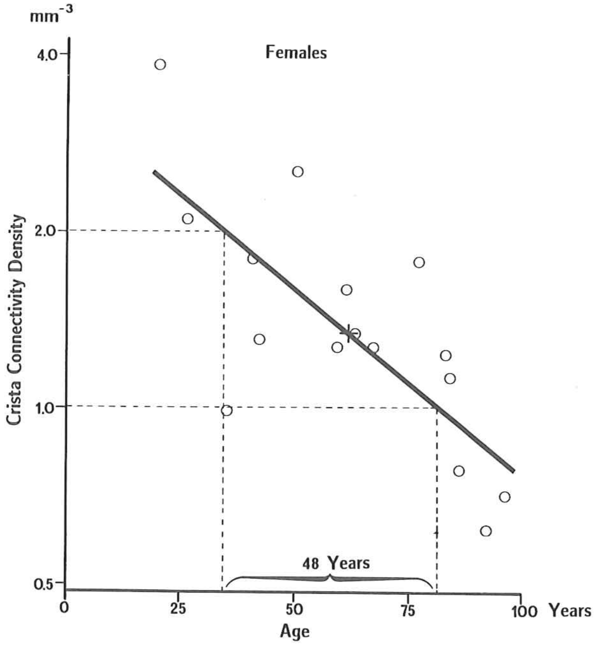

Fig. 3. Trabecular connectivity density as a function of age in femals, r = −0.74, 2p = 0.001. As indicated, the slope corresponds to a halving of trabecular number over about 50 years.

As expected, a reduction in bone volume fraction with age was found (data not shown), this reduction was significant only in females. However, as shown in Fig. 4, V_V(bone/tissue) depends only marginally on W_V(trabecular/tissue) and V_V cannot in general be used as a 'predictor' of connectivity. There is a tendency for the relation to be positive in females (r = 0.45, 2p = 0.078) but negative in males (r = −0.14, 2p = 0.58).

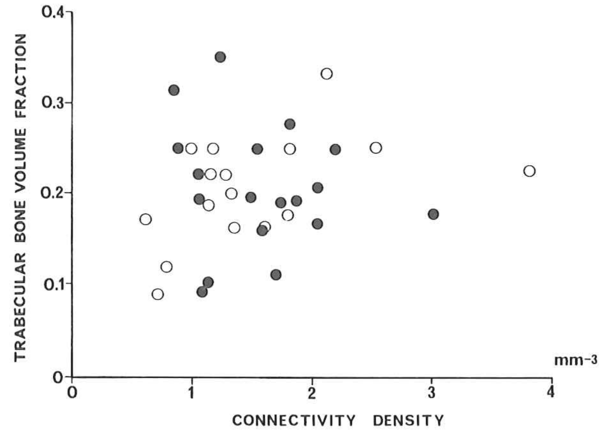

Fig. 4. The relationship between V_V(bone/tissue) and W_V(trabeculae/tissue). Open circles indicate females, filled circles indicate males. The relationship is not significant in either sex.

DISCUSSION

This study illustrates that connectivity density can be determined in a practical way on human iliac cancellous bone biopsies. The ConnEulor technique provides a simple and rapid means for obtaining connectivity density estimates in cancellous bone. Systematic random sectioning of the biopsies is not required, thus utilizing only approximately 500μm of the biopsy core. These results have significant implications for the routine implementation of this measurement in clinical biopsy specimens.

Previous studies have suggested that the pattern of bone loss and microstructural alterations in cancellous bone associated with aging differ between men and women. Connectivity changes are thought to occur primarily in women around the menopause due to imbalances in remodeling secondary to estrogen deficiency. The microstructural changes in men are considered to be characterized primarily by trabecular thinning. (Compston et al., 1987, Mellish et al., 1989, Aaron et al., 1987, Parfitt, 1988). The changes in connectivity density in this collection of iliac necropsies support the hypothesis that reductions in connectivity are a prominent feature of the age-related changes in cancellous bone in women. The loss in connectivity in women appears to be roughly parallel to the reductions in bone mass. Contrarily, in men, alterations in connectivity do not appear to be a major component of age-related microstructural changes. In men, the loss in bone mass appears to exceed the loss of connectivity. These differences between men and women are likely a consequence of differences in the pathogenesis of the remodeling imbalances (Parfitt, 1988).

REFERENCES

Aaron JE, Makins NB, Sagreiya K. The micronantomy of trabecular bone loss in normal aging men and women. *Clin Orthop Related Res.* 1987; 215: 260-271.

Compston JE, Mellish RW, Garrahan NJ. Age-related changes in iliac crest trabecular microanatomic bone structure in man. *Bone.* 1987; 8: 289-292.

Dempster DW, Shane E, Horbert W, Lindsay R. A simple method for correlative light and scanning electron microscopy of human iliac crest bone biopsies: qualities observations in normal and osteoporotic subjects. *J Bone Mineral Res.* 1986; 1: 15-21.

Gundersen HJG, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A, West MJ. The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS.* 1988; 96: 857-881.

Gundersen HJG, Boyce RW, Nyengaard JR, Odgaard A. The ConnEulor: unbiased estimation of connectivity using physical disectors under projection. *Bone.* 1993; 14: 217-222.

Mellish RW, Ferguson-Pell MW, Cochran GV, Lindsay R, Dempster, DW. A new manual method for assessing two-dimensional cancellous bone structure: comparison between iliac crest and lumbar vertebrae. *J Bone Mineral Res.* 1991; 6: 689-696.

Mellish RW, Garrahan NJ, Compston JE. Age-related changes in trabecular width and spacing in human iliac crest biopsies. ***Bone Miner.*** 1989; 6: 331-338.

Mosekilde Li. Consequences of the remodeling process for vertebral bone structure – a scanning electron microscopy study. ***Bone Miner.*** 1990; 10: 13-33.

Mosekilde Li, Mosekilde Lo, Danielsen CC. Biomechanical competence of vertebral bone in relation to ash-density and age in normal individuals. ***Bone.*** 1987; 6: 79-85.

Parfitt AM. Bone remodeling: relationship to the amount and structure of bone, and the pathogenesis and prevention of fractures. ***In: Riggs, B.L. & Melton, L.J. (ed), Osteoporosis: etiology, diagnosis, and management, Raven Press, NY,*** 1988; pp 45-94.

Parfitt AM. Age-related structural changes in trabecular and cortical bone: cellular mechanisms and biomechanical consequences. ***Calcif Tissue Int.*** 1984; 36: 9123-9128.

Parfitt AM, Mathews CHE, Villaneuva AR, Kleerekoper M, Frame B, Rao DS. Relationship between surface, volume, and thickness of iliac trabecular bone in aging and osteoporosis: implication for the micronantomic and cellular mechanisms of bone loss. ***J Clin Invest.*** 1983; 72: 1396-1409.

Recker RR, Kimmel DB, Parfitt AM, Davies KM, Keshawarz N, Hinders S. Static and tetracycline-based bone histomorphometric data from 34 normal postmenopausal females. ***J Bone Mineral Res.*** 1988; 3: 133-144.

Schenk RK, Olah AJ, Herrman W. Preparation of calcified tissues for light microscopy. ***In: Dickson, G.R. (ed). Methods of calcified Tissue Preparation, Elsevier, Ny,*** 1984; pp 1-56.

Sterio DC. The unbiased estimation of number and sizes of arbitary particles using the disector. ***J Microsc.*** 1984; 134: 127-136.