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STRUCTURAL ROLE OF BONE APATITE IN HUMAN FEMORAL COMPACTA

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Tensile and compressive strength of human femoral compacta have been shown to be related ($P > 0.005$) to the average bone apatite crystallite length (D_{002}) as determined by X-ray diffraction line breadth measurement. However, statistical variance of crystallite length was not sufficient to explain observed differences in mechanical properties, these differences being primarily due to variation in mineral density. Average bone apatite crystallite length was not found to change significantly with biological age ($P = 0.30$) over the range $3\frac{1}{2}$ to 87 years. It is concluded that increased bone apatite crystallite length is detrimental to the structural role of the skeleton but that this is not a major factor in determining fracture incidence in the elderly.

Key words: bone apatite; crystallite length; mechanical properties

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Compact bone is a composite material comprising collagen fibrils set in a non-collagenous matrix and incorporating calcium hydroxyapatite-like mineral inclusions (Wainwright et al. 1976). In addition, Posner (1969) has reported the presence of mineral in the form of amorphous calcium phosphate in bone. Correlation of the mechanical properties of bone tissue with mineral content is well established (Vose & Kubala 1959, Vose et al. 1961, Mather 1968, Currey 1969, Currey & Butler 1975), and increased incidence of bone fracture in the elderly (Knowelden et al. 1964) has been related to a decline in bone mineral content with age (Mulligan et al. 1975). Despite evidence of age-related change in bone apatite crystallite length and orientation (Chatterji & Jeffery 1968, Chatterji et al. 1972), the structural role of apatite in compact bone has not been directly investigated.

We have used X-ray diffraction line breadth measurement (Holmstrand 1957) to assess average bone apatite crystallite length (D_{002}) in human bone samples of differing biological age. Tensile and compressive mechanical properties were measured in the same bones and considered in relation to crystallite length.

MATERIALS AND METHODS

Human compacta excised post-mortem from 14 males and 15 females, ranging from 3½ to 87 years of age, was studied. All samples were removed from the proximal half of the femoral shaft, placed in polythene bags to prevent drying, and stored at −20°C until required. Separate portions were designated for the preparation of tensile and compressive test-pieces and X-ray diffraction powder samples. A steel template was used to prepare tensile test-pieces 3.1 mm thick and with a "waisted" portion 35 mm in length and 3.8 mm in width. Cylindrical compressive test-pieces 5.1 mm in diameter and 7.5 mm in length were produced with a diamond tipped core drill. Where the bone was of sufficient size, mechanical test-pieces were produced from four cross-sectional quadrants; this proved possible with 27 bones in the case of compressive test-pieces, and with 24 bones in the case of tensile test-pieces. Mechanical test-pieces remained moist when tested to destruction at a strain rate of 10^{-3} s^{-1}, as described in an earlier publication (Smith & Smith 1976a). The values of each tensile or compressive property determined for the four quadrants in each bone were averaged to reduce variation due to regional differences.

X-ray diffraction bone powder samples were produced by crude milling and subsequent wet-grinding with isopropyl alcohol in a micronizing mill. Grinding for 2 minutes produced a powder with particle size in the range 50–150 micron. The minimum particle dimension was large enough to ensure that the surface layer, which might contain crystallites ground to a size sufficiently small to produce X-ray diffraction line broadening, comprised less than one per cent of particle volume (Smith 1975). The line profile of the bone apatite (002) reflection obtained using nickel-filtered copper K_α radiation was monitored with a Philips vertical goniometer Type PW 1140. The goniometer was fitted with 1° divergence and scatter slits and a 1 mm receiving slit, and the scintillation detector was rotated at $\frac{1}{8}°$ 2θ per minute. Instrumental line broadening produced by this experimental arrangement was measured by recording the (111) reflection for each of ten NaCl standard powder samples possessing crystallite size too large (50–150 micron) to contribute to line broadening.

Calculations

Bone apatite (002) X-ray diffraction line profiles were shown to be described accurately ($r \geqslant 0.99$) by the Cauchy equation: $y = 1/(1 + K^2X^2)$. Observed line breadth at half peak intensity was corrected for broadening due to wavelength spread of the copper K_α doublet by interpolation from the standard data of Alexander & Klug (Alexander & Klug 1950, 1954). The mean "K_α – corrected" value of NaCl (111) line breadth represented an accurate estimate of instrumental line broadening at a diffraction angle close to that of the bone apatite (002) reflection. Hence, bone apatite (002) pure line breadth (β_{002}) was calculated by the method of Alexander & Klug (1950).

Pure line breadth results from small crystallite size and lattice strain (Warren & Averbach 1950). Harper & Posner (1966) have claimed that bone contains amorphous calcium phosphate (ACP), which causes diffuse X-ray scattering and gives rise to additional line broadening. However, ACP converts rapidly to hydroxyapatite in the presence of the precipitating solution (Termine et al. 1970), and there is no satisfactory theory to explain why this should not occur *in vivo*. The authors (Smith & Smith 1976b) do not believe that bone contains a substantial quantity of ACP, and Posner et al. (1975) now even express some uncertainty as to the short-term presence of ACP in bone as a chemical precursor in the formation of bone apatite *in vivo*.

Moreover, calculation based on accepted values for the rate of bone turnover in the adult (Villaneuva et al. 1966) shows that if ACP is precipitated in bone and converts to hydroxyapatite within 24 hours, then the proportion of amorphous bone mineral will be less than 0.03 per cent of the total, and would not significantly affect X-ray diffraction line breadth.

It is known that the Cauchy form of pure line profile, as observed in our investigation, is characteristic of pure line broadening due to small crystallite size (Posner et al. 1965, Schoening 1965). Thus, an estimate of bone apatite average crystallite size (D_{002}) normal to the (002) planes, can be calculated from the Scherrer equation:

$$D_{hkl} = C.\lambda / \beta_{hkl} \cos\theta_{hkl}$$

where λ is the intensity-weighted mean wavelength of X-radiation, θ_{hkl} is the Bragg angle for the (hkl) planes, β_{hkl} is the pure line breadth measured in radians, and C is a constant dependent upon the definition of β and the crystallites shape distribution. Absolute determination of D_{hkl} is inaccurate due to uncertainty as to the value of C, although Bragg (1919) has shown that if β is defined as the pure line breadth at half maximum intensity, then C takes a value of approximately 0.9 and is relatively insensitive to differences in shape. This value of C was assumed in the present investigation. Since the bone apatite c-axis has been shown to be parallel to the long axis of the crystallite (Engstrom & Finean 1953), D_{002} is equal to the average crystallite length.

RESULTS

The mean estimate of D_{002} obtained for all samples studied was 26.1 ± 0.65 nanometre ($P \geqslant 0.95$). Normal regression analysis revealed significant negative linear regression ($P < 0.005$) of both ultimate tensile and ultimate compressive stress, on D_{002}. Regression of tensile ($P < 0.0005$) and compressive ($P < 0.0025$) stress at the limit of proportionality, on D_{002} (Figures 1, 2), was also significant. No significant regression of D_{002} on age of the subject (Figure 3) was observed ($P = 0.30$), although crystallite impurity may have limited observed variance in D_{002}. Large crystallites are usually less pure than small ones (Chatterji & Jeffery 1968) and lattice distortion could have partially replaced crystallite size as a cause of line broadening.

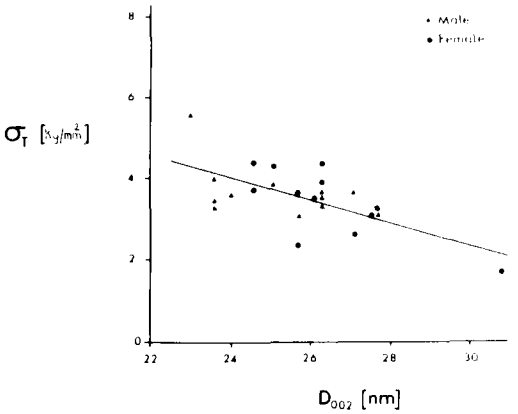

Figure 1. Least squares linear regression (correlation coefficient $r = -0.65$) of tensile stress at the limit of proportionality (σ_T), on average bone apatite crystallite length (D_{002}).

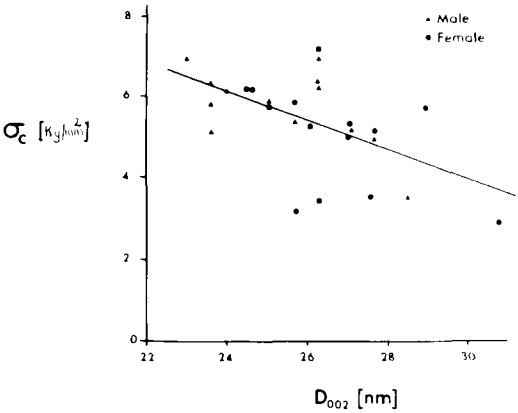

Figure 2. Least squares linear regression (correlation coefficient $r = -0.54$) of compressive stress at the limit of proportionality (σ_C), on average bone apatite crystallite length.

DISCUSSION

Bone apatite crystallites are reported to be elongated, with the long axis varying from 3 nanometre to 130 nanometre (Posner 1969, Lundy & Eanes 1973). Our mean estimate of 26.1 nanometre is consistent with X-ray diffraction measurement by other researchers when the effect of crystal lattice strain was not separately assessed (Carlstrom 1955, Wallgren 1957, Posner et al. 1963). Chatterji & Jeffery (1968) have proposed that crystallite size increases with age of the subject, and that this increase is a causal factor in bone fracture in the elderly. A theoretical basis for this hypothesis is provided by knowledge of the reinforcing role normally fulfilled by orientated elastic inclusions in composite materials (Biggs 1966). When such a composite is mechanically stressed in the fibre direction, the differing elastic properties of inclusion and matrix materials result in different strain of these two components. This in turn produces shear stress across each inclusion, the magnitude of shear stress being related to the length of the inclusion. The composite is effective in resisting

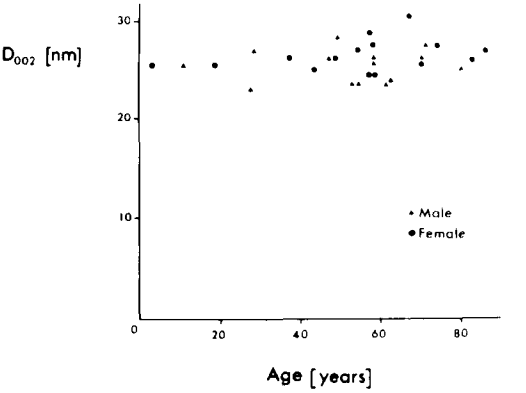

Figure 3. Average bone apatite crystallite length (D_{002}) versus biological age.

fracture if inclusions are long enough, and sufficiently numerous, to ensure that a crack may only propagate by traversing and thus fracturing a large number of them. However, if inclusions exceed a critical length determined by the relative elastic moduli of inclusion and matrix materials, then the breaking stress of each inclusion will be attained when a comparatively low stress exists in the surrounding matrix and cracks will readily propagate.

The present investigation indicates that ageing does not produce significant change in the length of bone apatite crystallites, and therefore that the high incidence of bone fracture in the elderly cannot be attributed to large crystallite length. Although Chatterji & Jeffery (1968) appeared to find evidence of increasing crystallite length with age, they investigated specimens from only three subjects. Moreover, their technique of treating the bone surface with enzymes, and examination by scanning electron microscopy, makes the results difficult to interpret (Wainwright et al. 1976).

The observed association between bone apatite crystallite length and mechanical properties of compact bone strongly supports the suggestion that apatite fulfils a reinforcing role and inhibits crack propagation. However, statistical variance of average crystallite length is insufficient to explain differences in the mechanical properties of bones studied. Indeed, a previous investigation (Smith & Smith 1976a) has shown that mineral density is the principal determinant of bone strength. It remains to be established whether greatly increased crystallite length might occur in metabolic bone disease, thus contributing to bone fracture in affected subjects.

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