Properties of growing trabecular ovine bone

PART II: ARCHITECTURAL AND MECHANICAL PROPERTIES

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We aimed to highlight the relationship between age and the architectural properties of trabecular bone, to outline the patterns in which the variations in these properties take place, and to investigate the influence of the architecture on the mechanical properties of trabecular bone in growing animals. We studied 30 lambs in three age groups and 20 sheep in two age groups. Cubes of subchondral bone were cut from the proximal tibia according to a standardised protocol. They were serially sectioned and their architectural properties were determined. Similar cubes were obtained from the identical anatomical position of the contralateral tibia and their compressive mechanical properties measured. The values obtained from the skeletally immature and mature individuals were compared. Multiple regression analyses were performed between the architectural and the mechanical properties.

The bone volume fraction, the mean trabecular volume, the architectural and the mechanical anisotropy, the elastic modulus, the bone strength, the energy absorption to failure, and the elastic energy correlated positively with increasing age whereas the connectivity density, the bone surface density, the ultimate strain, the absorption of viscoelastic energy and the relative loss of energy correlated inversely. The values of all variables were significantly different in the skeletally mature and immature groups. We determined the patterns in which the variations took place. The bone volume fraction of the trabecular bone tissue was found to be the major predictor of its compressive mechanical properties. Together with the mean trabecular volume and the bone surface density,

it explained 81% of the variations in the compressive elastic modulus of specimens obtained from the contralateral tibiae.

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Investigation of the architectural properties of trabecular bone using direct three-dimensional methods is relatively recent and is still at the stage of adjustment, refinement and comparison of the different methods. Variations in the architectural properties with skeletal growth have not been studied and information about skeletally immature individuals is not available. Previously, three-dimensional information has been obtained by stereological methods in which two-dimensional sectioning was used. These methods are highly effective but assumptions regarding regularity have to be made to estimate the topographical properties. Also, two-dimensional stereological methods cannot estimate the architectural anisotropy without making extensive simplifying geometrical assumptions.^{1,2}

It has been postulated solely on clinical observation and experience that the bone tissue of skeletally immature individuals behaves in a different way from that of adults and that the degree of anisotropy of trabecular bone may change with increasing weight-bearing. Our aims were to highlight the relationship between age and the architectural properties of trabecular bone in normal subjects, to outline the patterns in which the variations in these properties take place and to investigate their influence on the mechanical characteristics of trabecular bone in normal growing individuals.

Materials and Methods

We used trabecular bone from proximal ovine tibiae. The details are given in part I³ of this paper. One hind limb chosen randomly from each animal was used to determine the architectural properties. A 6 mm cube of subchondral trabecular bone was cut from the centre of the medial condyle of each tibia. Analysis of the reconstructed images of the cube was limited to $230 \times 230 \times 230$ voxels of 18 μm , that is a cube of bone with dimensions of 4.14 mm. This was to avoid potential errors connected with determin-

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ing the trabecular orientation at the border of the specimen. The protocol for bone cuts has already been described in detail in part I.³

The specimens were cleaned of bone marrow and degreased as described in part I.³ They were then bleached by immersion in 1% H₂O₂ for two hours to whiten the trabeculae so that there was sufficient contrast between them and the embedding medium (black). Lastly, they were air-dried at room temperature for 24 hours. The technique and the apparatus for serial sectioning and three-dimensional reconstruction have already been described in detail by Odgaard et al.^{4,5}

Specimens from the contralateral tibiae were obtained from identical anatomical positions and prepared and mechanically tested as described in part I.³

The architectural variables considered were as follows:

Bone volume fraction (BV/TV). The volume (%) of bone (BV) in a specimen divided by its total volume (TV).

Connectivity density (CN). The total number of trabeculae (Euler number ± 1) (trabeculae/mm³) in a specimen divided by its volume in mm³.

Mean trabecular volume (MTV). The total bone volume of a specimen (mm³) divided by its total number of trabeculae.

Bone surface density (BS/TV). The surface area (mm²/mm³) of the trabeculae (BS) in relation to the total volume (TV) of the specimen.

Volume orientation (VO). The local volume orientation is defined for any point within the bone as the orientation of the longest intercept within the point in question (Fig. 1).

Architectural anisotropy (AA). The ratio between the longest and the shortest diameter of the ellipsoid derived from the analyses of the volume orientation.

Statistical analysis. The statistical analysis and the stepwise multiple regression model were performed in the same way as described in part I.³

Fig. 1

Diagram illustrating the definition of the volume orientation in a trabecular bone cube. At each randomly placed point the local volume orientation is indicated by the direction of the longest intercept passing through the point.

Results

Architectural properties

Table I gives the mean and the SEM of the different variables in the different age-groups and Table II gives the corresponding values for the skeletally immature and the skeletally mature groups.

Figure 2 illustrates two examples of the reconstructed specimens, one from the youngest and one from the oldest age groups.

Bone volume fraction (BV/TV). The mean BV/TV of the specimens obtained from the skeletally immature group was 22% lower than that of the skeletally mature group, which is statistically significant (Table II). The Kruskal-Wallis test showed that there was a significant difference between the group means ($p = 0.004$). Pairwise analysis

Table I. The mean (SEM) of the different variables (see text) and sample size in the five age groups

Group	BV/TV	CN	MTV	BS/TV	AA (VO)
I	30.6 (1.4)	9.76 (0.81)	0.033 (0.002)	2.50 (0.06)	2.33 (0.20)
II	35.2 (2.3)	8.33 (0.80)	0.046 (0.005)	2.56 (0.06)	2.99 (0.25)
III	34.4 (2.7)	8.81 (0.68)	0.039 (0.005)	2.46 (0.08)	2.22 (0.09)
IV	37.2 (2.9)	4.99 (0.38)	0.082 (0.013)	2.23 (0.04)	3.46 (0.29)
V	46.4 (3.2)	3.01 (0.25)	0.168 (0.022)	2.09 (0.05)	3.25 (0.31)

Table II. Mean (SEM) of the different variables in the skeletally immature (groups I to III) and skeletally mature (groups IV and V)

	Skeletally immature	Skeletally mature	p value*
Bone volume fraction (%)	32.7 (1.3)	41.8 (2.4)	0.001
Connectivity density (trabec/mm ³)	8.97 (0.44)	4.00 (0.32)	< 0.001
Mean trabecular volume (mm ³)	0.039 (0.003)	0.125 (0.016)	< 0.001
Bone surface density (mm ² /mm ³)	2.50 (0.04)	2.16 (0.04)	< 0.001
Architectural anisotropy (volume orientation)	2.52 (0.12)	3.35 (0.21)	< 0.001

* Mann-Whitney U test

Table III. A summary of the results of analysis of variance, including pairwise comparisons (Mann-Whitney U test). Significant p values are in bold

	p value*				
	BV/TV	CN	MTV	BS/TV	AA (VO)
Kruskal-Wallis test	0.004	< 0.001	< 0.001	< 0.001	< 0.001
Mann-Whitney U test					
Group I v group II	0.186	0.082	0.049	0.496	0.026
Group I v group III	0.762	0.096	0.545	0.990	0.970
Group I v group IV	0.104	< 0.001	< 0.001	0.003	0.003
Group I v group V	< 0.001	< 0.001	< 0.001	< 0.001	0.019
Group II v group III	0.364	0.427	0.290	0.385	0.003
Group II v group IV	0.521	< 0.001	0.019	< 0.001	0.227
Group II v group V	0.014	< 0.001	< 0.001	< 0.001	0.676
Group III v group IV	0.199	< 0.001	0.004	0.019	< 0.001
Group III v group V	0.005	< 0.001	< 0.001	0.005	0.005
Group IV v group V	0.049	0.002	0.007	0.070	0.545

* BV/TV, bone volume fraction (%); CN, connectivity density (trabec/mm³); MTV, mean trabecular volume (mm³); BS/TV, bone surface density (mm²/mm³); AA, architectural anisotropy; and VO, volume orientation

between the groups (Mann-Whitney U test, Table III) showed that the differences were mainly between the three skeletally immature groups (groups I, II and III) and the oldest group (group V). No significant difference was found among the skeletally immature groups, while the difference between the two skeletally mature groups showed a borderline significance ($p = 0.049$). This means that volume fraction increased slowly during the period of growth, reached a peak at maturity, and further slowly increased in adulthood and throughout the period of observation. A significant positive correlation was found between BV/TV and increasing age (Spearman's correlation, $\rho = 0.56$, $p < 0.001$). The correlation was not statistically significant when the skeletally immature individuals were considered separately.

Connectivity density. The mean value of the CN in the skeletally immature group was twice as high as that in the mature group (Table II). This difference was statistically significant (Mann-Whitney U test, $p < 0.001$). Analysis of variance showed that the means in the five age groups

differed significantly from each other (Kruskal-Wallis test, $p < 0.001$). Pairwise analyses between the groups (Mann-Whitney U test, Table III) showed that the differences were between all the skeletally immature and the two skeletally mature groups. The three skeletally immature groups showed no mutually significant differences, while the two skeletally mature groups did. This means that the CN decreased slowly during the period of growth and further decreased during adulthood. There was a significant inverse correlation between the CN and increasing age (Spearman's correlation, $\rho = -0.77$, $p < 0.001$). Within the skeletally immature group the correlation was not significant.

Mean trabecular volume (MTV). The mean value in the skeletally immature individuals was three times lower than that of the skeletally mature group (Mann-Whitney test, $p < 0.001$, Table II). The means according to age group are shown in Table I with the most minimal differences being in the three skeletally immature groups. Analysis of variance between the means of the five age groups showed a statistically significant difference (Kruskal-Wallis test,

Fig. 2a

Fig. 2b

Diagrams of examples of reconstructed specimens a) from the youngest and b) from the oldest age groups showing the differences in the trabecular number and thickness.

Fig. 3a

Fig. 3b

Diagrams showing the main direction of trabecular orientation in a) the skeletally immature ($n = 30$), and b) the skeletally mature animals ($n = 20$). The circle represents a globe seen from above in which 90° is the anatomical anterior direction. The points represent the main trabecular direction in each specimen. The ellipse is the 99% confidence interval of the mean of the main trabecular direction in the sample. Co-ordinates for the mean are: a) $X = 0.090$, $Y = -0.566$, $Z = 0.824$; and b) $X = 0.134$, $Y = 0.704$, $Z = 0.697$.

Table III). Results of pairwise comparisons between the groups are shown in Table III. The pattern was simply a slow increase in the MTV throughout the period of skeletal maturation and then a marked increase during adulthood. There was a statistically significant positive correlation between MTV and increasing age (Spearman's correlation, $\rho = 0.75$, $p < 0.001$). The correlation was not significant when the skeletally immature animals were considered separately.

Bone surface density (BS/TV). The skeletally immature animals had values which were 14% higher than the skeletally mature, which was statistically significant (Mann-Whitney U test, Table II). Analysis of variance showed a significant difference between the group means (Kruskal-Wallis test, $p < 0.001$). Significant differences were seen exclusively between the skeletally immature (groups I, II and III) and the skeletally mature (groups IV and V, Table III). This means that the decrease was mainly during the period of growth. There was a statistically significant inverse correlation between the BS/TV and increasing age (Spearman's correlation, $\rho = -0.67$, $p < 0.001$). When the skeletally immature animals were considered separately there was no significant correlation with age.

Architectural anisotropy (AA). This was calculated from the volume orientation analysis. The mean of the main direction of orientation of the trabeculae of the skeletally immature animals was inclined posteriorly whereas that of the skeletally mature was anteriorly. Figure 3 shows the mean of the main direction of orientation in the skeletally mature and the skeletally immature animals, together with the 99% confidence interval (CI) of the mean of the two samples. This could not be tested statistically in a classical way, but the 99% CIs for both groups are very far apart corresponding to a classical p value of less than 1% ($p < 0.01$). Figure 4 shows the distribution of the mean

direction of the ellipsoid derived from volume orientation data in the five age groups. There is a gradual change in the main direction of orientation with increasing age, but more material is needed to quantify the differences between the five age groups. The AA was 25% lower in the skeletally immature than in the skeletally mature animals which was significant (Mann-Whitney U test, Table II). Analysis of variance showed a significant difference between means for the five age groups (Kruskal-Wallis test, Table III). Pairwise analyses between the groups indicated that the AA increased during the period of growth and that no further significant changes were observed after maturity. A positive significant correlation between the AA and increasing age

Fig. 4

A diagram showing the mean of the main direction of trabecular orientation in the five age groups. The figures I to V represent points through which passes the line representing the mean value of the main direction of trabecular orientation for each age group. There is a gradual change in the main trabecular direction of orientation with age.

was observed (Spearman's correlation, $\rho = 0.39$, $p = 0.007$).

The effect of body-weight. The body-weight of the animal is significantly correlated with age (Spearman's correlation $\rho = 0.80$, $p < 0.001$). When age was substituted for body-weight as the independent variable against the other investigated variables, similar patterns of correlation were obtained.

The effect of gender on the properties of trabecular bone. None of the studied architectural properties was significantly affected by gender.

Architectural and mechanical properties. The multiple regression model (see part I)³ showed that the BV/TV was the major explanatory variable of the dependent mechanical variables. Introduction of the different architectural variables significantly increased the explanatory power of the model. As a last step 'age' was introduced as an independent factor, which proved to have an effect on the explanatory power of the model regarding absorption of viscoelastic energy. The results of the multiple regression model were of course negatively affected by the fact that the data sets of the mechanical variables were derived from specimens obtained from the contralateral limb. Table IV shows the explainable portion of the variables of the different compressive mechanical properties as a function of the explanatory variables, together with the level of significance at each step of the forward stepwise multiple regression model.

Discussion

There are no data available in the literature concerning either the architectural or the mechanical properties of trabecular bone in skeletally immature individuals. Previously, three-dimensional (3D) information has been obtained from stereological methods, in which two-dimensional (2D) sectioning was used. Whitehouse,⁶ Harrigan et al,⁷ Cowin,^{8,9} Cowin and Mehrabadi¹⁰ and Turner and Cowin¹¹ developed the theoretical foundation for these structure-property relationships. Finite-element analysis plays a significant role in the 3D analysis and comprehension of the properties of architectural bone. Nevertheless,

the method is highly dependent on the accuracy of the input of the data, which in turn reflects the accuracy of the data obtained from 3D reconstruction of the object in question. Excellent examples of this combination of experimental and analytical method are illustrated by the studies of Kabel et al¹² and Van Rietbergen et al.¹³ In 1990 Odgaard et al⁴ introduced a new, direct method to obtain 3D information on an openly connected porous structure, based on serial sectioning and 3D reconstruction. It is unbiased, offers a high resolution at a low cost, and is the one which we used. With the progress of micro CT and micro MRI other 3D non-destructive methods with more or less the same high resolution are now becoming commercially available, but at a higher cost.

The architectural properties of bone have been explored in relation to age, aiming mostly at changes related to ageing, and using almost exclusively 2D analytical techniques.¹⁴⁻²¹ The relationship between the architectural and the mechanical properties of bone has been studied by several authors, using both 2D^{2,6,14-17,19,21-24} and 3D approaches.^{12,13,25-31} So far, none of the available quantitative 3D reports describes the patterns in which variations in the architectural properties of bone in young individuals take place.

The variations in the architectural variables observed in our study indicate a dynamic process occurring at different velocities throughout the period of growth. Thus, the BV/TV, the MTV and the AA increased with increasing age, whereas the CN and the BS/TV decreased.

Fukuda and Iida¹⁴ studied the architectural properties of trabecular bone of 90 growing Beagle dogs aged from 3 to 24 months using histomorphometric measurements. They reported an increase in the BV/TV and mean trabecular thickness from three to nine months of age. They also reported that the BS/TV increased up to the age of nine months, then decreased until maturity (aged 24 months). These patterns of variation in relation to age are almost identical with our findings.

Studies have shown that the BV/TV plays a major role in the determination of the mechanical properties of trabecular bone.^{12,30,32} Behrens et al,³² in their study on human trabecular bone obtained from the knee, reported a sig-

Table IV. The result of forward stepwise multiple regression analysis. The portion (percentage) of the mechanical properties which could be explained out of the architectural properties is given. The p values are given in parentheses

	Percentage explainable*				
	E	ϵ_u	δ_θ	ϵ_v	δ_v
Bone volume fraction (%)	57 (<0.001)	59 (<0.001)	NS	NS	NS
Mean trabecular volume (mm^3)	62 (<0.001)	NS	31 (<0.001)	13 (0.009)	NS
Architectural anisotropy (VO)	NS	63 (<0.001)	38 (<0.001)	NS	10 (0.017)
Bone surface density (mm^2/mm^3)	81 (<0.001)	66 (<0.001)	54 (<0.001)	NS	NS
Connectivity density (trabec./ mm^3)	NS	NS	NS	NS	NS
Age in months	NS	NS	NS	NS	18 (0.044)

* E , elastic modulus (MPa); σ_u , ultimate stress (MPa); W_u , energy absorption to failure (kJ/m^3); ϵ_u , ultimate strain (%); δ_v , viscoelastic energy absorption (kJ/m^3); and VO, volume orientation

nificant correlation between the BV/TV and bone strength. Vesterby et al³⁰ reported similar results in a study of human vertebral trabecular bone.

Several authors have described a decrease in the BV/TV with increasing age.^{15,17,20,21} In all of these studies the age span was from young to very old and probably explains the disagreement between these reports and our findings. Hodgkinson and Currey^{22,23} studied human and bovine trabecular bone using a 2D approach. They were able to explain up to 93% of the variations occurring in the elastic modulus using three architectural properties (fabric, BV/TV and CN) along with the apparent density. This combination of variables seems peculiar, first because the apparent density correlates highly with BV/TV and secondly because we have found that the portion of CN participating in the multiple regression model is negligible. Kabel et al¹² studied specimens of trabecular bone obtained at post-mortem from six different anatomical locations in 55 human donors. They found that the mechanical properties of trabecular bone can be predicted with high precision (89%), using two architectural bone properties, namely BV/TV and the fabric mathematical relationship developed by Cowin.⁸

Goldstein et al²⁶ and Goulet et al²⁷ studied the mechanical and the architectural properties of cubes of human trabecular bone using 3D micro CT. The cubes were obtained from four donors aged from 31 to 69 years, and were taken from seven different metaphyseal regions. They found that the elastic modulus correlated positively with the BV/TV and with CN. We have confirmed the positive correlation between the elastic modulus and BV/TV, but for the CN our findings showed an inverse correlation with the elastic modulus. This is probably explained by the different connectivity measurements performed, and the nature of the material of the two studies. The decrease in CN and mean trabecular volume with increasing age which we have found agrees with other reports.^{17,18}

The conflicting findings regarding the relationship of CN with other properties of bone, strongly suggest that it plays a minor role, if any, in determining the elastic properties of trabecular bone. Besides, it reflects the controversial status of the significance of CN as a determinant of the mechanical properties of bone when it is not as unambiguous as commonly perceived. The BS/TV, however, which is intuitively dependent upon the trabecular number, is an important explanatory factor of the variations in the compressive elastic properties. While CN is a quality of trabecular bone characterised by being independent of the trabecular shape or orientation in space, the BS/TV is dependent on both the number and volume of trabeculae. Hence, it is more likely that this double dependency makes the BS/TV an active factor in explaining the mechanical properties. In an editorial Compston³³ agreed that the CN was less likely to play a major role in the mechanical competence of trabecular bone, and urged research workers to perform further studies to explore this point.

Our findings have shown that the AA correlated positively with increasing age.^{34,35} This is significant in explaining the variations which occur in the compressive strength and in the energy absorption of trabecular bone.^{26,27} Korstjens et al^{34,35} examined the trabecular pattern and orientation at different locations within the distal radius of children aged from 4 to 14 years using a radiological technique. They concluded that the degree of anisotropy at a location just proximal to the epiphyseal plate increases during the period of growth.

Our finding of decreasing trabecular number together with increasing AA with age, suggests that the decrease in the trabecular number mainly takes place in a plane perpendicular to the main trabecular direction, confirming some earlier reports,^{1,28,36,37} but contradicting others.^{20,38} The age-related gradual changes in the main direction of trabecular orientation which we found probably contributes to the comprehension of these controversies.

We found that gender had no effect on the variables which agrees well with earlier reports.^{23,39}

It is very difficult to compare the findings of previous studies with our results because of several factors such as the model (human/animal of different species), the age-span of the different materials, the anatomical site of sampling, the methods of obtaining the architectural variables (2D and 3D methods), and not least the different definitions of the different variables. Based on our observations, and parallel to what was stated in part I,³ we can say that in order to withstand the increasing demands on the bone tissue the organism forms more bone tissue thus increasing the volume fraction, changes the trabecular number and morphology, and changes the trabecular orientation in space. The last are reflected in changes occurring in the main directional orientation of the trabeculae and in the AA. Our findings show differences in the properties of trabecular bone between skeletally immature and mature animals and offer the patterns in which the changes occur during the period of growth. They also provide relationships between the different properties of trabecular bone in an animal model. These facts highlight the need for exploring these patterns and relationships in human tissues.

We conclude that the architectural properties of trabecular bone differ significantly with skeletal maturity. Compared with that of skeletally mature animals the trabecular bone of the skeletally immature is less dense, contains a greater number of thinner trabeculae per unit volume, and is less anisotropic. The main direction of trabecular orientation changes significantly with increasing age. The architectural properties significantly affect the compressive mechanical properties of trabecular bone. The BV/TV, the CN, the mean trabecular volume and, to some extent, the AA are the major variables which determine the compressive elastic constants of trabecular bone. Connectivity does not correlate positively with the elastic modulus as has been commonly perceived.

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