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Age variations in the properties of human tibial trabecular bone and cartilage

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To my family

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List of papers

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I. **Ding M, Dalstra M, Danielsen CC, Kabel J, Hvid I, Linde F.** Age variations in the properties of human tibial trabecular bone. *J Bone Joint Surg (Br)* 1997; 79-B: 995-1002.

*II. **Ding M, Odgaard A, Linde F, Hvid I.** Age-related variations in the microstructure of human tibial cancellous bone. 2000; Submitted for publication.

III. **Ding M, Dalstra M, Linde F, Hvid I.** Mechanical properties of the normal human tibial cartilage-bone complex in relation to age. *Clinic Biomech* 1998a; 13: 351-358.

IV. **Ding M, Dalstra M, Linde F, Hvid I.** Changes in the stiffness of the human tibial cartilage-bone complex in early-stage osteoarthrosis. *Acta Orthop Scand* 1998b; 69: 358-362.

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Structure of the thesis:

This review concentrates on two related fields: 1) normal age-related variations in the mechanical, physical/compositional, and structural properties of trabecular bone, and 2) age- and osteoarthrosis-related changes in mechanical properties of the cartilage-bone complex. The review consists of an appraisal of recent investigations and own studies: study I to study IV (Ding et al., 1997; Ding et al., 1998a; Ding et al., 1998b; Ding et al., 2000). Furthermore, recent investigations on the properties of two most common age-related musculoskeletal degenerative diseases—osteoarthrosis and osteoporosis are summarized in the discussion section. The summary and conclusion based on own studies are presented, and suggestions for future study are also proposed.

Introduction

1.1 General introduction

Age-related variations in the properties of trabecular bone and articular cartilage have always been central issues for both basic researchers and clinicians. There is an increase in the incidence of age-related musculoskeletal diseases, such as osteoarthrosis (OA) (Kirwan and Silman, 1987), and osteoporosis (OP) (Riggs, 1991; Steiniche, 1995), and an increase in the incidence of fragility fracture (Martin, 1993; Mosekilde, 1993), as a result of increase in the elderly population and a change in lifestyle. These diseases are among the major health care problems in terms of socio-economic costs. Therefore, a thorough understanding of age-related variations in the properties of trabecular bone and articular cartilage is crucial for diagnosis, prophylaxis, and treatment of the age-related musculoskeletal diseases, and for design, fixation and durability of joint prosthesis.

The normal individual reaches peak bone mass at age between 25 and 35 years, and thereafter bone mass declines with age in both sexes (Melsen et al., 1978). However, the bone loss patterns differ for men and women. In men, decreased formation may be the principal factor, and bone loss attributes to generalized attenuation of trabecular bone. In women, increased resorption seems to be the principal factor, and bone loss attributes to the total removal of individual trabeculae. These different patterns of bone loss suggest differences in bone remodeling between both sexes as a consequence of aging (Aaron et al., 1987).

The bone loss acceleration is accompanied by structural change. Reduction in bone mass and change in structure may result in a reduction of mechanical strength in both sexes. These reductions in strength and bone mass are disproportionate, i.e. the reduction in mechanical strength has a greater extent than bone tissue loss itself would suggest (Kleerekoper et al., 1985; Parfitt et al., 1983; Parfitt, 1987). In addition, trabecular bone becomes increasingly brittle and fractures with less energy. It has been hypothesized that this tendency is driven by the need for remodeling to repair fatigue damage, while most remodeling events fail to replace all the bone that they remove (Martin, 1993).

Bone remodeling affects bone collagen fiber orientation, architectural anisotropy, connectivity, mineralization degree, and the amount of unrepaired fatigue damage, which are additional determinants of bone strength and stiffness. The relation between structure and mechanical strength has attracted increasing interest, as studies have clearly documented that mechanical strength depends not only on the bone mass, but also on bone structure (Kleerekoper et al., 1985). Thus, the knowledge of three-dimensional (3-D) structural properties of trabecular bone is essential for a better understanding of the age-related variations of bone tissue.

Age-related decline in the mechanical properties of human articular cartilage differs between anatomic locations (Kempson, 1991). For the femoral head, the tensile fracture stress of articular cartilage declines for both superficial and mid-depth zones. For the femoral condyles of the knee, the tensile strength and stiffness of the superficial zone increase with age to a maximal value in the third decade, thereafter decline significantly with increasing age, while the tensile strength and stiffness from the deep zone decline steadily with age (Kempson, 1991).

It is assumed that the deterioration in the tensile properties of the collagen fiber network results in the decrease in tensile properties of cartilage. The progressive fatigue failure and the cartilage structural change are due to altered chondrocyte metabolism resulting in the reduction in tensile properties with age (Kempson, 1991). Thus, the structure of cartilage may play a more important role than the composition in determination of its mechanical properties (Guilak et al., 1994).

1.1.1 Properties of normal trabecular bone

1) Mechanical properties of trabecular bone

Many investigations have been carried out on the mechanical properties of trabecular bone, and the associations among them (Carter and Hayes, 1977; Currey, 1979; Dalstra et al., 1993; Goldstein et al., 1991; Hvid and Hansen, 1985; Hvid, 1988; Hvid, 1988; Hvid et al., 1989; Keaveny and Hayes, 1993; Keaveny et al., 1994b; Linde and Hvid, 1989; Linde et al., 1989; Linde et al., 1991; Odgaard et al., 1989; Odgaard and Linde, 1991; Røhl et al., 1991). The age-related variations in these properties have also been described (Bell et al., 1967; Lindahl, 1976; Mosekilde et al., 1987; Weaver and Chalmers, 1966). Young's modulus (normalized stiffness) and failure energy have been found to be inversely linearly correlated with age.

For the last two decades, studies have mainly focused on central vertebral trabecular bone with two exceptions (Lindahl, 1976; McCalden et al., 1993). Compressive strength from human femoral trabecular bone has been reported to decrease by 8.5% per decade (McCalden et al., 1993). The elastic modulus and ultimate stress for tibial trabecular bone have been shown to relate inversely with age (Lindahl, 1976). However, dried defatted specimens were investigated in this latter study (Lindahl, 1976), a method of preparation that tends to alter the mechanical properties (Carter and Hayes, 1977; Linde and Sorensen, 1993).

Age-related variations in the properties of human cortical bone have been investigated (Burstein et al., 1976; Currey and Butler, 1975; Currey, 1979; McCalden et al., 1993). Elastic modulus and bending strength were found to increase until about 30 years, and decrease thereafter (Currey, 1979). It has also been reported that strength, strain and failure energy are inversely linearly correlated with age (McCalden et al., 1993).

2) Physical properties of trabecular bone

Densities, such as apparent density and volume fraction, have been most intensively investigated and often used as predictors for mechanical strength and elastic modulus of trabecular bone. Density alone can largely explain the trabecular bone strength and elastic modulus (Ciarelli et al., 1991; Goulet et al., 1994).

Several investigations have been carried out on the contribution of mineral and collagen, the major components of bone, to the mechanical properties of cortical bone. The results of Burstein et al (Burstein et al., 1975) indicate that strength and elastic stiffness depend on the mineral content, whereas the plasticity of bone is a function of the properties of collagen. It has been shown that collagen orientation is an important factor in determining mechanical properties of cortical bone (Martin and Ishida, 1989; Martin and Boardman, 1993). Whether this parameter also has similar effect for determining mechanical properties of trabecular bone remains to be seen.

Despite many investigations on apparent density and apparent ash density in relation to age, the relative importance of collagen and mineral in determining the mechanical properties of trabecular bone has attracted little attention (Mosekilde et al., 1987; Riggs et al., 1981; Weaver and Chalmers, 1966). Age-related variation in collagen content and its influence on mechanical properties have been investigated in animal models (Danielsen et al., 1986; Danielsen et al., 1993). There are apparently no published studies on the age-related variation in human collagen density, one major compositional parameter of trabecular bone.

3) Structural properties of trabecular bone

Investigations have shown significant age-related changes in structural parameters of trabecular bone, such as volume fraction, surface density (McCalden et al., 1997; Mosekilde, 1988; Mosekilde, 1989; Parfitt et al., 1983; Thomsen et al., 1998), and bone surface-to-volume ratio (Mosekilde, 1988; Mosekilde, 1989; Parfitt et al., 1983; Thomsen et al., 1998). Investigations have also shown a significant age-related decrease in the thickness of horizontal trabeculae, but not in the thickness of vertical trabeculae (Mosekilde, 1988), a gender-dependence of connectivity (Thomsen et al., 1998), bone volume (Aaron et al., 1987), and a significant age-related increase of the marrow space star volume (Vesterby et al., 1991b).

These available quantitative data on age-related changes in trabecular structure were either based on histomorphometric technique (Aaron et al., 1987; Mosekilde, 1988; Mosekilde, 1989), or on model-based two-dimensional (2-D) approaches (Compston et al., 1987; McCalden et al., 1997; Parfitt et al., 1983; Parfitt, 1984). These results are unbiased and reliable only if the assumed model is not violated. In contrast to model-based 2-D methods, by applying the theorems of stereology on histological sections (Boyce et al., 1995; Thomsen et al., 1998; Vesterby, 1993), it is possible to obtain unbiased and reliable results. Nevertheless, no direct examination of age-related changes in 3-D microstructure of cancellous bone has been published.

Age-related studies on trabecular structure have mainly focused on central vertebral trabecular bone (Mosekilde, 1988; Mosekilde, 1989; Mosekilde, 1998; Vesterby et al., 1991a), and iliac crest trabecular bone (Aaron et al., 1987; Compston et al., 1987; Parfitt et al., 1983), but one study includes distal femoral trabecular bone (McCalden et al., 1997). However, the method used in this latter study (McCalden et al., 1997) was based on the plate model, a method that has been recognized to be subject to serious limitations (Feldkamp et al., 1989; Odgaard, 1997). Therefore, no general conclusions have been established for age-related variations in the microstructure of human peripheral trabecular bone.

Anisotropy, connectivity and density (volume fraction) are considered as the primary important characteristics in describing trabecular bone architecture (Odgaard, 1997). Some investigators reported that bone volume fraction and architectural anisotropy accounted for 68-90% (Goulet et al., 1994), for more than 80% (Goldstein et al., 1993), or for more than 90% (Odgaard et al., 1997; van Rietbergen et al., 1998b) of the variance in the elastic modulus and ultimate strength. Density alone can explain 40–80% of the variation of elastic modulus and strength (Ciarelli et al., 1991). It has been hypothesized that a primary reason for decreasing strength and stiffness in osteoporosis is loss of trabecular elements and consequently a loss in connectivity (Kleerekoper et al., 1985; Mosekilde, 1989; Parfitt, 1987).

Architectural anisotropy, orientation of trabeculae, has been quantified by different methods. The mean intercept length (MIL) method is an interface-based method (Whitehouse, 1974), whereas the volume orientation (VO) method is representative of volume-based methods (Odgaard et al., 1990; Odgaard et al., 1997). It has been claimed that volume-based methods predict mechanical properties better than surface-based methods (Odgaard et al., 1997; van Rietbergen et al., 1998b).

Connectivity is a fundamental property of 3-D networks. Traditionally, connectivity has been measured by various surrogate 2-D methods, however, no known relation of these methods exists to 3-D connectivity (Odgaard and Gundersen, 1993). Unbiased and model-free quantification of connectivity from 3-D images is enabled by a topological approach, and this approach also allows quantification of the mean size of individual trabecula and the mean volume of marrow space (Gundersen et al., 1993; Odgaard and Gundersen, 1993).

The age-related variations in the most important parameters, such as architectural anisotropy and connectivity, of peripheral trabecular bone are poorly understood. Until now, no study on the age-related variations in 3-D microstructure of human peripheral (tibial) trabecular bone has been published.

1.1.2 Properties of osteoarthrotic and osteoporotic trabecular bone

OA and OP are two of the most common, age-related diseases. They are rarely both seen in the same patient (Dequeker, 1985; Dequeker, 1997). Clinical and epidemiological investigations have suggested a negative association between them (Cooper et al., 1991). However, no clear relationship between them was found.

1) Properties of osteoarthrotic trabecular bone

OA is characterized by articular cartilage degeneration, subchondral bone sclerosis and cysts, osteophyte formation, and joint space narrowing. OA has become one of the major health care problems in western countries. OA stands alongside cancer and heart disease as one of the major causes of suffering and disability amongst the elderly (Bailey and Mansell, 1997). OA is basically a joint disease but it is difficult to define more precisely. Research into the etiology of OA has for many decades focused on articular cartilage destruction. The changes in the proteoglycans are believed to initiate the disease, and subsequently changes in the supporting collagenous framework, thereafter the disease becomes irreversible (Bailey and Mansell, 1997).

OA may cause deterioration of the stiffness of subchondral cancellous bone (Ding et al., 1998b), while the stiffness of subchondral bone plate may increase due to the increase in density. Nevertheless, this increase in stiffness is much less than the increase in density itself would suggest (Li and Aspden, 1997b). OA also results in structural changes of trabecular bone. It is generally known that an increase occurs in volume fraction and trabecular thickness (Kamibayashi et al., 1995b). However, changes in architectural anisotropy and connectivity in different OA stages are largely unknown.

Despite research efforts, the etiology of OA is still unknown. It is generally agreed to be multifactorial. Recent investigations support the hypothesis that subchondral bone plays a significant role in the cartilage degeneration in OA (Burr, 1998). Whether the initial changes in OA occur first in cartilage or first in the subchondral trabecular bone remains unproven. Studies on the properties of cartilage and subchondral bone have always been done either on cartilage or on subchondral bone alone.

2) Properties of osteoporotic trabecular bone

OP is characterized by low bone mass, microstructural deterioration of bone tissue enhancing bone fragility, and increased in fracture risk (Consensus development conference, 1991). OP is a bone disease affecting mainly women after the menopause when estrogen deficiency predisposes to accelerated bone loss. OP is categorized as primary and secondary. The primary OP includes type I, postmenopausal OP and type II, senile OP. Secondary OP is characterized by identifiable causal factors other than menopause and aging, i.e. OP is caused by sporadic causal factors, such as endocrine disorders, gastrointestinal disease, and corticosteroid-therapy etc (Gennari et al., 1998; Steiniche, 1995; Vaananen, 1991).

OP is a major public health problem (Avioli, 1988). Genetic, hormonal, and environmental elements are the widely accepted important factors in the pathogenesis of OP. These factors directly affect peak bone mass, perimenopausal bone loss and age-related bone loss (Eriksen and Langdahl, 1997; Ralston, 1997). Peak bone mass is mainly genetically determined, although dietary calcium, and physical activity can also have a positive effects (Eriksen and Langdahl, 1997; Sambrook et al., 1993). The peak bone mass is higher in men than women since men have bigger bones, while peak bone mineral density is the same (Seeman, 1997). By age 80, women have lost approximately 40% of their peak bone mass and men 25% (Riggs, 1991).

Bone loss, however, is not the sole factor affecting bone strength. Bone strength may also be impaired by changes in bone geometry, microstructure, and quality that occur along with loss of bone mass (Frost, 1985). For cortical bone, decrease in the thickness and increase in the porosity of cortical bone compromise its strength. For trabecular bone, the loss of bone results in thinning, perforation and removal of entire internal supporting structures. Bone loss, mainly of trabecular bone, with its fragility fracture is an unavoidable consequence of OP. These fractures mainly occur in vertebrae, distal radius, and proximal femur.

To summarize the background, investigations on the properties of trabecular bone have for at least the past two decades been focused on the central vertebral, femoral or iliac trabecular bone. As a consequence, many articles have been published on the properties of central vertebral and iliac trabecular bone (Mosekilde, 1993; Mosekilde, 1998; Steiniche, 1995). These investigations provide a better understanding of the age-related changes in the properties of central trabecular bone. However, the age-related variations in the properties of peripheral trabecular bone are relatively unknown.

1.1.3 Properties of normal articular cartilage

1) Properties of normal articular cartilage

The joint is an organ rather than a structure. Synovial joints—the functional connections—control the motion between bones. Approximately 0.1 to 5 mm thick cartilage covers the articulating bony ends, this depending on species and anatomical location (Athanasiou et al., 1991). The solid matrix (20–30% of the wet weight of the cartilage) and the interstitial fluid (70–80%) constitute articular cartilage. The solid matrix is composed of collagen fibers (65% of dry weight), proteoglycans (25%), other glycoproteins, lipids and chondrocytes (Muir, 1983). The remaining 70–80% of cartilage is water, most of which can exchange freely with the outside medium (Mow et al., 1980).

Articular cartilage is a structure rather than a material. The mechanical properties of articular cartilage depend on the complex structure and interactions of its biochemical constituents. Articular cartilage is viscoelastic, which is observed in the deformational responses under many loading conditions. This is primarily due to fluid flow through the solid matrix (Cohen et al., 1998). It seems that microstructural changes, rather than compositional changes, of the collagen-proteoglycan solid matrix are central events. These changes are responsible for the early increase of hydration and the deterioration of mechanical properties of articular cartilage (Setton et al., 1993), and in the progression of OA (Guilak et al., 1994).

It is clear that proteoglycan loss from the solid matrix will alter the physicochemical properties of the tissue. However, it is still not quite clear which pathological processes and biochemical mechanisms will lead to this loss. The age-related degeneration of articular cartilage is often related to a breakdown of the normal load-bearing capacity of cartilage. Several factors may lead to such a breakdown: direct trauma to the cartilage, obesity, immobilization, and excessive repetitive loading of the cartilage.

2) Mechanical testing of articular cartilage

The most commonly used modes of mechanical testing of articular cartilage are confined compression creep testing (Armstrong and Mow, 1982; Hayes and Mockros, 1971), unconfined compression testing (Camosso and Marotti, 1962), shear testing (Hayes and Mockros, 1971), indentation testing (Mow et al., 1984), and tensile testing (Kempson, 1991). Based on these techniques, age-related variations in the mechanical properties of human cartilage have been investigated on the femoral head (Kempson, 1991), the femoral condyle (Kempson, 1982; Swann and Seedhom, 1993), the tibial condyle (Swann and Seedhom, 1993), the patella (Armstrong and Mow, 1982), and the talus (Kempson, 1991; Swann and Seedhom, 1993).

Despite enormous amounts of published data on the mechanical properties of both cartilage and bone, however, all mechanical testing of these tissues have been done separately. Although articular cartilage and underlying trabecular bone might function as a mechanical unit *in vivo*, little is known about the mechanical behavior of both tissues when they are tested simultaneously.

A novel technique developed by Røhl et al. (1997) for simultaneous measurement of mechanical properties of articular cartilage and subchondral trabecular bone has enabled a direct investigation of mechanical properties of cartilage and bone.

1.1.4 Relations between osteoarthrotic cartilage and underlying trabecular bone

Previous investigations on the variation in the stiffness of articular cartilage in different species, different joints and different locations within the joint have shown the same pattern (Armstrong and Mow, 1982; Mow et al., 1991) as that of trabecular bone (Hodgskinson and Currey, 1990; Hvid and Hansen, 1985). This relationship suggests that cartilage and subchondral trabecular bone may function as a mechanical unit. However, this hypothesis has never been rigorously tested. No matter how we assume the initiation of OA being "cartilage first" or "bone first", both hypotheses suggest the disruption of unit function of the cartilage-bone complex. Nevertheless, no published study is available aiming at investigating the mechanical properties of OA cartilage and subchondral trabecular bone simultaneously.

1.1.5 Definitions for the properties of trabecular bone and articular cartilage

1) Mechanical properties

Any material with a linear elastic component under uniaxial loading can be described by a proportional relationship between change in load and change in length of the material. During mechani-

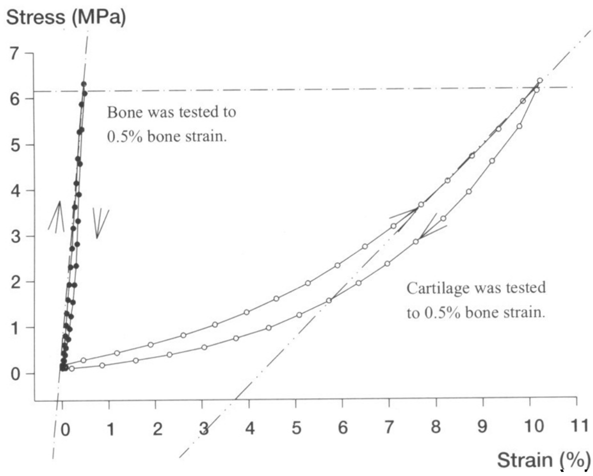

Figure 1. A typical combined testing stress-strain curve (Study III & IV), both cartilage and bone were tested non-destructively to 0.5% bone strain.

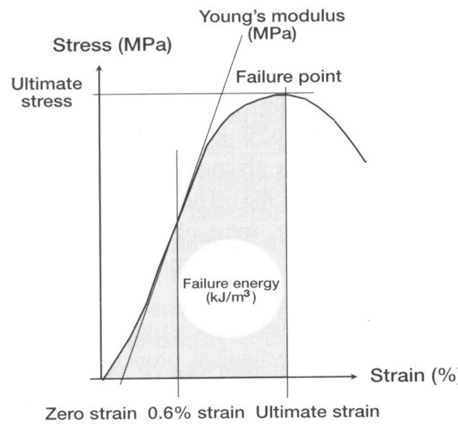

Figure 2. A typical stress-strain curve from a destructive test (study I). Ultimate stress and ultimate strain were derived from the first maximum of the curve, Young's modulus was determined as the tangent to the point on the loading curve intersecting the 0.6% strain line, failure energy as the area underneath the compression curve between zero strain and ultimate strain.

cal testing, a force-deformation curve is obtained. This curve can be converted to stress and strain curve using the cross-sectional area of the specimen for normalization of load to stress and the original length of the specimen for normalization of deformation to strain. Two typical mechanical testing curves are shown in Figures 1 and 2.

- *Young's modulus (elastic modulus, or normalized stiffness):* Reflects the stiffness of a material, determined from both non-destructive and destructive testings (Figures 1 and 2), and calculated as the tangent to the loading curve of the stress-strain diagram at a given axial strain. Unit: MPa.
- *Ultimate stress (strength):* Determined from destructive testing (Figure 2), and calculated from the first point with maximal stress. Unit: MPa.
- *Ultimate strain:* Determined from destructive testing (Figure 2), and calculated from the first point with maximal stress. Unit: percent.
- *Failure energy:* Determined from destructive testing (Figure 2), and calculated as the area underneath the compression curve between zero strain and ultimate strain. Unit: kJ/m^3.
- *Elastic energy capacity:* Determined from non-destructive testing (Figure 1), and calculated from the area under the unloading curve. Unit: kJ/m^3
- *Viscoelastic energy absorption:* Determined from non-destructive testing (Figure 1), and calculated from the area enclosed by the loading-unloading loop. Unit: kJ/m^3.
- *Relative energy loss (loss tangent):* Determined from non-destructive testing (Figure 1), and calculated as Viscoelastic energy/(Viscoelastic + Elastic energies). Unit: percent.

2) Physical/compositional properties

- *Tissue density:* Reflects the mineralized bone tissue (material) density, determined from Archimedes' principle, and calculated as dry weight of the specimen divided by the volume of bone matrix excluding marrow space. Unit: g/cm^3.
- *Apparent density:* Reflects the mineralized bone apparent (structural) density, and calculated as dry weight of the specimen divided by the volume of specimen. Unit: g/cm^3
- *Apparent ash density:* Reflects the mineralized bone non-organic density, and calculated as ash weight of the specimen divided by the volume of specimen. Unit: g/cm^3
- *Collagen density:* Reflects the bone organic

density, and calculated as total collagen weight divided by the volume of specimen. Unit: g/cm^3

- *Collagen concentration:* Calculated as the total collagen weight of the specimen divided by the total dry weight of the specimen. Unit: percent.
- *Mineral concentration:* Calculated as the total mineral content (ash weight) of the specimen divided by the total dry weight of the specimen. Unit: percent.

3) Three-dimensional structural properties

- *Architectural anisotropy:* Reflects orientation of trabeculae, namely, its main direction and the degree of dispersion around it, quantified based on a 3-D volume method: star volume distribution (SVD). Degree of anisotropy is calculated as the eigenvalue of the primary direction divided by the eigenvalue of the tertiary direction. Unit: dimensionless.
- *Connectivity density:* Reflects the fundamental property of 3-D networks, and quantified in an unbiased and model-free manner by the Euler number from 3-D images using a topological approach. Connectivity density is standardized by specimen volume. Unit: mm^{-3}.
- *Volume fraction:* Calculated as bone volume of a specimen divided by its total volume. Unit: percent.
- *Bone surface density:* Calculated as bone surface area of a specimen divided by its total volume. Unit: mm^{-1}.
- *Bone surface-to-volume ratio:* Calculated as bone surface area of a specimen divided by bone volume of the specimen. Unit: mm^{-1}.
- *Mean trabecular volume:* Calculated as the total bone volume of the specimen divided by its "connectivity+1". Unit: μm^3.
- *Mean marrow space volume:* Calculated as the marrow space volume of the specimen divided by its "connectivity+1". Unit: μm^3.

1.2 Purpose of the study

The aims of the present study concentrated on two aspects: age-related variations in the properties of human peripheral (tibial) trabecular bone (studies I & II), and age- and osteoarthrosis-related changes in the mechanical properties of cartilage-bone complex (studies III & IV).

• ***Study I***

1) to investigate normal age-related variations in the mechanical properties, and physical/compositional properties of human tibial trabecular bone; and 2) to examine the associations among them, especially the associations between collagen, mineral and mechanical properties.

• ***Study II***

1) to investigate normal age-related variations in the 3-D microstructural properties of human tibial trabecular bone, with specific emphasis on architectural anisotropy and connectivity; 2) to assess whether age-related trends in the properties differ for the medial and the lateral condyles.

• ***Study III***

1) to investigate the age-related variations in the mechanical properties of cartilage-bone complex, and 2) to assess the association between the articular cartilage and underlying trabecular bone.

• ***Study IV***

1) to compare the stiffness relations of the cartilage-bone complex in early-stage osteoarthrosis with normal age-matched group; and 2) to test the hypothesis that the unit function of cartilage and bone alters under pathological conditions.

Materials

2.1 Normal trabecular bone specimens (studies I & II)

Human autopsy proximal tibiae, one from each donor, without macroscopical pathological changes or a history of musculoskeletal diseases, were collected and stored at –20 °C. All the donors were Caucasian and they had been active on a normal level until two weeks before death.

For study I: Age variations in mechanical, and physical/compositional properties
Thirty-one tibiae were harvested from 31 donors, aged 16 to 83 years, 5 females and 26 males. Seven cylindrical trabecular bone specimens were produced from standardized locations of the medial condyle and six from the lateral condyle from each tibia (specimens no. 1–13, Figure 3) (Linde et al., 1988), yielding a total of 403 specimens.

For study II: Age variations in structural properties
Forty tibiae were retrieved from 40 donors, aged 16 to 85 years, 10 females and 30 males. Two randomly selected cylindrical trabecular bone specimens were obtained from both medial and lateral condyles (Figure 3), yielding a total of 160 specimens.

2.2 Normal cartilage-bone complex specimens (study III)

Cylindrical cartilage-bone complex specimens were produced from the same 31 normal tibiae as used in study I. From each tibia, one cylindrical cartilage-bone complex specimen was obtained from the standardized location on medial and lateral condyles where cartilage surfaces were most plane (medial: no. 2, and lateral: no. 12. Figure 3). Thus, a total of 62 specimens were produced. The testing of cartilage-bone complex was first conducted before sectioning bone from complex specimen for use in study I.

2.3 Osteoarthrotic cartilage-bone complex specimens (study IV)

Cylindrical cartilage-bone complex specimens were removed from 9 human early-stage OA proximal tibiae [7 males, 2 females, mean age 74 (63–81) years], and 10 normal human age-matched proximal tibiae [7 males, 3 females, mean age 73 (60–85) years]. All these donors were Caucasian. One tibia was removed from each donor, and 3 specimens were taken from each medial and lateral condyle (medial: no. 2, 3, 6, and lateral: no. 9, 12, 13, Figure 3). These specimens were divided into 4 groups: medial OA (n=27), lateral comparison (n=27), medial age-matched (n=30), and lateral age-matched (n=30).

The normal tibiae were without macroscopical pathology or history of musculoskeletal diseases, and all the cartilage surfaces of the specimens were intact. These patients had all died suddenly from trauma or acute disease.

The early-stage OA was defined as macroscopically degenerated fibrillated cartilage, and was confirmed histologically, according to criteria described by Mankin et al. (1971). The OA tibiae showed visual degeneration with slight fissures on the surface (superficial zone) of the medial

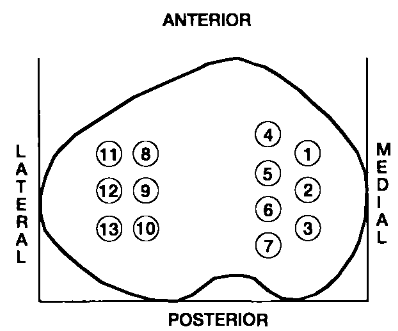

Figure 3. Schematic drawing illustrates a plastic template, which was available in different scales. They were used for obtaining specimens from the standardized locations of tibial condyles. The scale of the template was chosen according to the width of the slice. The template was aligned on the line formed by the posterior margin of both condyles. See text.

condyle cartilage, whereas the surface of the lateral condyle cartilage was intact. The cell clusters in the superficial zone and reduction of Safranin-O staining of OA cartilage were observed.

2.4 Specimen preparation

Using a trephine with an inner diameter of 7.5 mm, the cartilage-bone complex specimens were drilled out of frozen specimens. The orientation of the cylindrical specimens was such that the longitudinal axis of the tibia corresponded to the axis of the cylinder. After this, the distal part of trabecular bone was cut off, using a LEITZ Saw Microtome 1600 (Ernst Leitz Wetzlar Gmbh, Wetzlar, Germany) to achieve a bone length of 8.5 mm. The exact dimensions were afterwards measured using a caliper, and the mean value of three measurements from different locations around the periphery was used.

The trabecular bone specimens were cut 1 mm below the subchondral bone plate and at the distal end to obtain a specimen with a diameter and length of 7.5 mm. The exact dimensions were measured using a micrometer.

All the specimens were stored in sealed plastic tubes at –20 °C until testing.

Methods

3.1 Mechanical testing

3.1.1 Mechanical testing of bone

The mechanical testing of bone (study I) was performed on an INSTRON material testing machine (Model 4302, Instron Ltd., High Wycombe, Bucks, UK), using a 1 kN load cell and a static strain gauge extensometer (Model 2601-20, Instron Ltd.) attached to the testing column close to the specimen. The testing columns with polished ends were lubricated with low-viscosity mineral oil to reduce the effect of friction (Linde and Hvid, 1989). The testing machine was computer-controlled by Testpoint console software (Testpoint, Capital Equipment Co., Burlington, Massachusetts, USA) which also handled force and deformation data collection. The force-deformation data were converted to stress and strain data using the cross-sectional area of the specimens for normalization of load to stress and the original length of the specimens for normalization of deformation to strain.

Up to 10 preconditioning cycles were performed between a pre-load of 3 N (zero strain) and 0.6% strain to reach a viscoelastic steady state before the actual test was performed, only the actual test data being collected for analysis. Specimens used for non-destructive testing were tested with a strain rate of 0.002/sec. The Young's modulus (normalized stiffness) was obtained from non-destructive testing. Ultimate stress, ultimate strain and failure energy (energy absorption to failure) were obtained from destructive testing, using the same strain rate and preceded by mechanical conditioning as used for non-destructive testing (Figures 1 and 2).

3.1.2 Mechanical testing of cartilage-bone complex

The mechanical tests of cartilage-bone complex were performed on an INSTRON material testing machine (for study III) or on an 858 Bionix MTS hydraulic material testing machine (MTS Systems Coperation, Minneapolis, Minnesota, USA) (for study part IV), using a 1 kN load cell.

The mechanical set-up was the same in both testing machines. This set-up for combined testing was done as described by Røhl et al. (1997) (Figure 4). The specimen was mounted in an aluminum cage by 3 screws fixed tightly onto the subchondral bone plate. The cage had a broad mid-plate, which formed a reference plane between cartilage and bone. The cartilage was oriented downwards, and submerged in a bath filled with physiological saline. The top 2.5 mm of the lower column was made of porous sintered steel, with porosity 30% and a mean pore size of 20 μm to allow outflow of water from the cartilage.

The complex specimen was tested simultaneously by non-destructive compression to 0.5% bone strain, with a strain rate of 0.002 per second (Røhl et al., 1997). Deformations of the cartilage and the trabecular bone were measured simultaneously as the average between the data of the two lower strain transducers and the two upper strain transducers, respectively. For each testing, 20 preconditioning cycles were performed between 4 N and 0.5% bone strain to reach a viscoelastic steady state of bone before the actual test was performed, and only the actual test data were collected for analysis.

Force-deformation curves were recorded on a PC. The force-deformation data were converted to stress and strain data. The cross-sectional area of the specimen was used for normalization of load to stress and the original lengths of cartilage and bone were used for normalization of deformation to strain. From the test data, Young's moduli were determined for both tissues (Figure 1).

The Young's modulus of bone was determined as the tangent to the point on the bone-loading curve intersecting the 0.45% bone strain line. The elastic energy of bone was determined from the area underneath the bone-unloading curve between zero strain and 0.45% bone strain. Viscoelastic energy was determined from the area en-

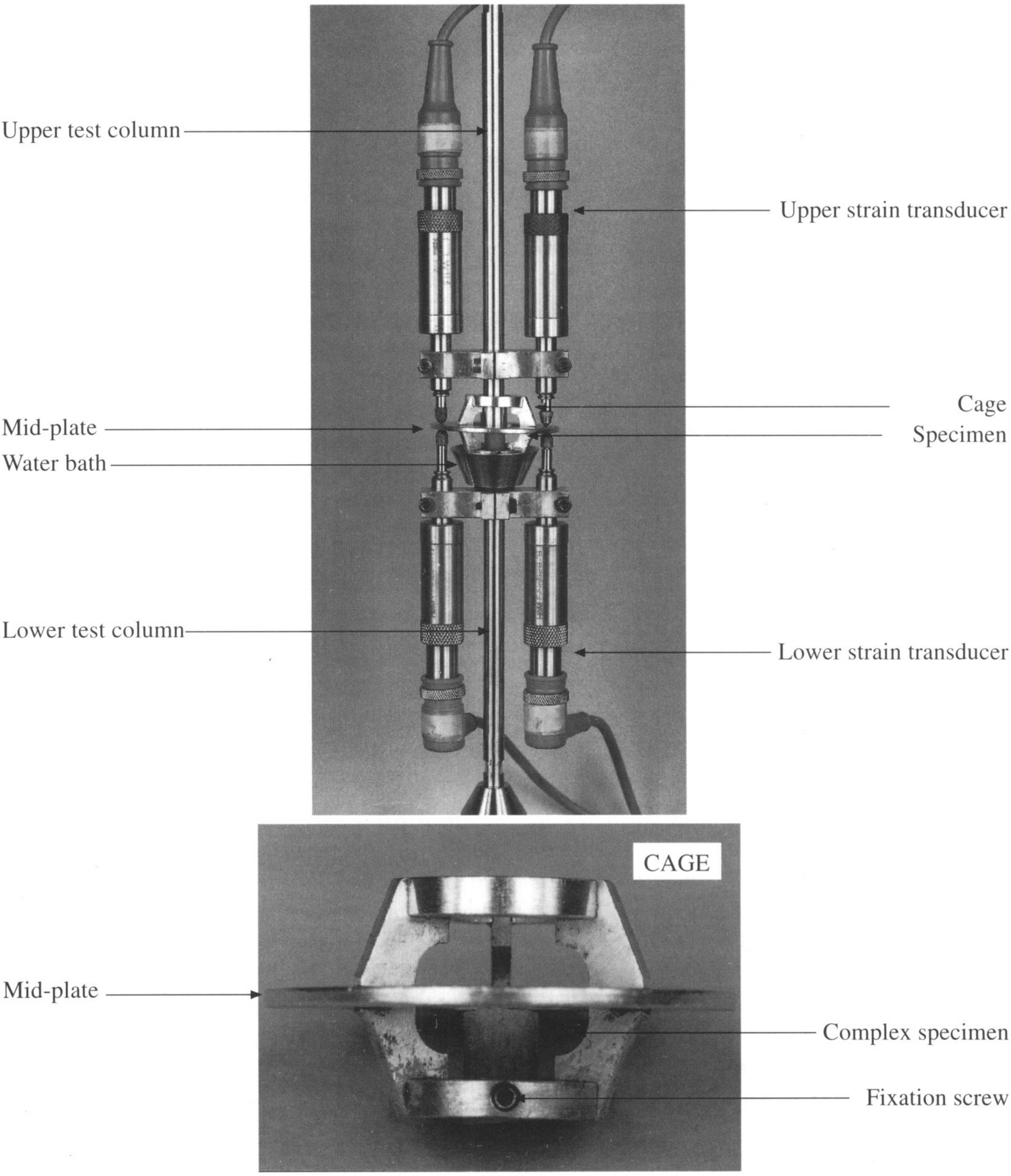

Figure 4. Mechanical set-up for combined testing (studies III & IV). The specimen was mounted in a cage by 3 screws fixed onto the subchondral bone plate. The specimens were tested in compression between two steel columns. Deformations of cartilage and bone were measured by two sets of strain transducers. See text.

closed by the loading-unloading loop. The relative energy loss was calculated as viscoelastic energy divided by the sum of viscoelastic and elastic energies (Linde, 1994).

The Young's modulus of cartilage was determined as the tangent to the point on the cartilage loading curve intersecting the cartilage strain line (corresponding 0.45% bone strain). The elastic energy of cartilage was determined from the area underneath the cartilage-unloading curve between zero strain and cartilage strain. The viscoelastic energy and relative energy loss of cartilage were determined like those of bone (Figure 1).

3.2 Physical parameter measurements

3.2.1 Density and volume fraction measurements

After testing, the marrow was removed from the specimens by air jet and tap water. The density and volume fraction were determined based on an accurate protocol (Sharp et al., 1990), with a slight modification. That is, the specimens were defatted in a much less toxic alcohol and acetone 1:1 mixture (in stead of trichlorethylene) for 48 hours. Then specimens were cleaned once more by air jet and tap water and then evaporated at room temperature for 24 h and freeze-dried.

Dry weight (DW) of the freeze-dried specimens was recorded. To determine the tissue density (ρ_{tiss}), each sample was rehydrated under vacuum in a Ringers solution adding a wetting agent (Pervitro 75%) and the submerged weight (μ_s) of the sample was recorded in a Mettler AT250 balance (Mettler Instruments AG, Greifensee, Switzerland) equipped with a density determination kit. Submerged weight and dry weight were determined twice. From the mean weights, the apparent density (ρ_{app}) was calculated from the dry weight of the defatted specimen divided by its original volume, and the tissue density was calculated as:

$$\rho_{tiss} = DW * \rho_L/(DW - \mu_s)$$

where $\rho_L = 1.00$ g/cm^3 is the density of the submersion liquid. Volume fraction (V_V) was derived as (ρ_{app} / ρ_{tiss}).

3.2.2 Collagen and mineral measurements

After this, the specimens were cleaved into 4 pieces for duplicate determinations of ash and collagen. In order to account for inhomogeneity of the specimens, duplicate determinations of ash and hydroxyproline were performed on both a distal and a proximal piece (Figure 5).

The collagen content of the specimens was estimated by measuring hydroxyproline, assuming 13.4% (w/w) hydroxyproline content in collagen (Neuman and Logan, 1950). After hydrolysis of the specimens in 6 M HCl at 100 °C for 16 h, the hydroxyproline was estimated according to the procedure of Woessner (1976) with slight modification of the concentration of reagents (Grant,

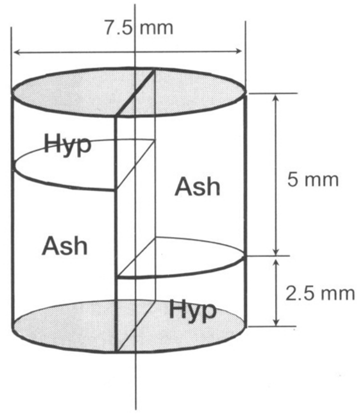

Figure 5. A schematic drawing showing the way specimens were cleaved, two major pieces were used for ash weight determination, two minor pieces were used for hydroxyproline (Hyp) determination.

1965) as described (Danielsen and Andreassen, 1988). The collagen (tissue) concentration was the amount of collagen divided by the dry weight, and the collagen (apparent) density was the collagen weight divided by specimen volume.

The bone specimens were ashed in a muffle oven at 100 °C for 2 h and 580 °C for 18 h, and dry weight of ash determined. The mineral concentration was the amount of mineral divided by dry weight, and the apparent ash density was the mineral weight divided by specimen volume.

3.3 Three-dimensional reconstruction

3.3.1 Microtomographic scanning

The specimens were scanned with a high resolution microtomographic system (μ-CT 20, Scanco Medical AG., Zürich, Switzerland). A detailed description of this system can be found elsewhere (Rüegsegger et al., 1996). Typically, measurement procedures are as follows: scout view, examination volume selection, automatic positioning, measurement, segmentation, reconstruction, and off-line 3-D evaluation and display. Scanning time for each specimen was approximately 3 hours. Our specimens were scanned in a standard resolution and multislice mode, resulting in a voxel size of x-, y-, and z-direction of 22 x 22 x 22 mm^3. Each 3-D image data set consisted of approximately 350 micro-CT slide images (512 x 512 pixels) with 16-bit-gray-levels. Each 3-D image data

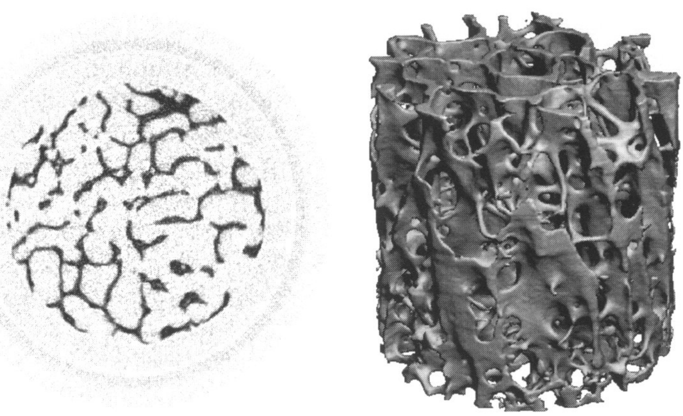

Figure 6. Typical micro-CT images of cylindrical cancellous bone specimens (left). After threshold procedure, 3-D reconstruction was made (right), which consists of approximately 350 image slices. All the structural parameters were calculated based on 3-D data sets.

set had a total size of 450 Mb unsegmented, image size of 76 Mb, and 38 Mb in segmented form.

3.3.2 Data segmentation with optimal threshold

After scanning, all micro-CT data were segmented using individual thresholds determined by the scanner-supplied algorithm. A significant deviation of volume fraction was found (2%, $p<0.05$) between micro-CT and Archimedes-based determinations. Relationships between the two determinations showed both the y-intercept and the slope of the regression line to be significantly different ($p<0.001$). This underestimation was corrected by applying new individual thresholds corresponding to the Archimedes-based volume fractions. Thus the accurate three-dimensional data sets were obtained from micro-CT images (Figure 6).

3.4 Structural properties based on 3-D methods

3.4.1 Anisotropy

A volume method: star volume distribution (SVD) was used to quantify architectural anisotropy, its main direction and the degree of dispersion around it (Cruz-Orive et al., 1992; Odgaard et al., 1990; Odgaard et al., 1997). The SVD describes the typical distribution of trabecular bone around a typical point in a trabecula. The result of SVD may also be expressed by a normalized fabric tensor, the SVD fabric tensor.

Each purified original micro-CT data set was cut to a central reduced size, which allowed the planar faces of this volume parallel to and 2 mm away from the original faces of the specimen. This procedure excluded any effect of artificial edges (Odgaard et al., 1997). The specific choice of 2 mm was made after considering the architecture of the cylindrical specimens, thus the reduced specimens had a diameter and length of 3.5 mm, still on a continuum level (Goulet and Hollister, 1996). The star volume distribution was determined in 3-D space for 500 random orientations for 300 random points within the trabeculae and a SVD fabric tensor was determined.

Eigenvalues of the fabric tensors were designated by τ_1, τ_2, and τ_3, where $\tau_3 < \tau_2 < \tau_1$. The fabric tensors were normalized by dividing by $\tau_1 + \tau_2 + \tau_3$, so that $\tau_1 + \tau_2 + \tau_3 = 1$. Here the eigenvalue τ_1 reflects the relative anisotropy strength of the primary direction, τ_2 the secondary direction, and τ_3 the tertiary direction. The eigenvectors corresponding to τ_1, τ_2, and τ_3 were designated $\mathbf{u}_1$, $\mathbf{u}_2$ and $\mathbf{u}_3$, respectively. $\mathbf{u}_1$ defines the primary direction, $\mathbf{u}_2$ the secondary and $\mathbf{u}_3$ the tertiary direction. These three directions are the main directions, which are orthogonal (Odgaard et al., 1997). The degree of anisotropy (DA) was defined as the eigenvalue of the primary direction divided by the eigenvalue of the tertiary direction (Goulet et al., 1994).

3.4.2 Connectivity, mean trabecular volume and mean marrow space volume

In order to quantify connectivity in an unbiased manner, the edge problem must be solved (Odgaard and Gundersen, 1993). The diameter and length of the 3-D data sets were reduced by 0.4 mm to produce a central reduced-size specimen with a diameter and length of 7.1 mm. The reduced set will be called I (interior), the exterior to this E. The total original specimen is hence $T = I \cup E$, and the interface between interior and exterior is $E \cap I$. The Euler numbers were calculated for the entire cylinder [$\chi(T)$], the interior [$\chi(I)$], and the exterior [$\chi(E)$]. The Euler number of the interface is calculated as (Odgaard and Gundersen, 1993).

$$\chi(I \cap E) = \chi(I) + \chi(E) - \chi(T) \quad (1)$$

The Euler number (χ) is the key to all determinations of connectivity and it is related to connectivity and the number of bone and marrow particles (Odgaard and Gundersen, 1993):

$$\chi = \beta_0 - \beta_1 + \beta_2 \quad (2)$$

where b_0 is the number of bone particles, b_1 is the connectivity and b_2 is the number of marrow cavities fully surrounded by bone. During the purification process of the entire specimen it is ensured that the bone structure is one connected component ($b_0(T) = 1$), and that no isolated marrow cavities exist ($b_2\ (T) = 0$) (Odgaard and Gundersen, 1993). By defining the reduced specimen (I), b_2 will remain 0 $(b_2(E) = b_2(I) = 0)$, but b_0 of I and E may easily be larger than one. Alternatively, b_0 and b_2 may be determined independently in the 3-D data set. Using equation (2), equation (1) may be reformulated into

$$\chi(I \cap E) = \beta_0(I) - \beta_1(I) + \beta_2(I) + \beta_0(E) - \beta_1(E) + \beta_2(E) - \beta_0(T) + \beta_1(T) - \beta_2(T) \quad (3)$$

and since $b_2(E) = b_2(I) = 0$ and $b_0\ (T) = 1$

$$\beta_1(\mathrm{I}) + \beta_1(\mathrm{E}) = \beta_1(\mathrm{T}) + \Delta(\beta_1) \quad (4)$$

where

$$\Delta(\beta_1) = \beta_0(\mathrm{I}) + \beta_0(E) - 1 - \chi(I \cap E) \quad (5)$$

The connectivity lost by dividing the total specimen T into an interior I and an exterior E part should be assigned equally to the interior and exterior parts in determining connectivity density and in calculating mean trabecular and marrow space volumes. Consequently, a corrected connectivity may be defined for the interior part as

$$\beta_1^*(\mathrm{I}) = \beta_1(\mathrm{I}) - 1/2\ \Delta(\beta_1) \quad (6)$$

$$= \beta_0(\mathrm{I}) - \chi(I) + \beta_2(\mathrm{I}) - 1/2\ (\beta_0(\mathrm{I}) + \beta_0(\mathrm{E}) - 1 - \chi(I \cap E)) \quad (7)$$

$$= 1/2 - \chi(I) + 1/2(\beta_0(\mathrm{I}) - \beta_0(\mathrm{E})) + 1/2\ \chi(I \cap E) \quad (8)$$

This may be compared to $\beta_1 = 1 - \chi$, which gives the relation between connectivity and the Euler number for a specimen, where $\beta_0 = 1$ and $\beta_2 = 0$. The connectivity density can now be calculated for the internal part I as

$$CD = \beta_1^*(I)/V(I) \quad (9)$$

where V (I) is the volume of the internal part. The mean trabecular volume *(MTV)* and the mean marrow space volume *(MMSV)* are given by

$$MTV = (V(I)^*Vv)\,/\,(\beta_1^*(I) + 1) \quad (10)$$

$$MMSV = (V(I)^*(1-Vv))\,/\,(\beta_1^*(I) + 1) \quad (11)$$

where Vv is the bone volume fraction in the internal part.

3.4.3 Volume fraction, bone surface, surface-to-volume ratio, and surface density

From the 3-D micro-CT data sets, the volume fraction (bone volume per total specimen volume) was determined in a cylindrical volume-of-interest, which was only slightly smaller than the specimen (0.1 mm margin excluded). Bone surface area in mm^2, bone surface-to-volume ratio in mm^{-1} (bone surface area per bone volume), and bone surface density in mm^{-1} (bone surface area per total volume of specimen) were also calculated from 3-D data sets.

3.5 Statistical analysis

Study I

The data were divided into young (16–39 years), middle (40–59 years) and old age groups (60–83 years). All the statistic analyses (SPSS, SPSS Inc. Chicago, Illinois, USA) were based on the entire data sets. The mean value for each tibia was used in analyses of age-related variations, and in analysis of multiple associations among various structural properties (each individual represented by one set of values), thus data are independent from one to another. For the property comparisons, only the same specimens that had been determined for the same properties were used.

One-way ANOVA was used to compare the properties in the three different age groups. These data were first checked for equal variance, and for normality. If the F-test showed a significant level, a multiple comparison was made by the Bonferroni test to find differences between groups. Then ANOVA was further performed based on age groups divided into decades to test for any significant difference among decades. Since there was only one tibia in the second decade group, this tibia was included in the third decade, defined as the younger age group (16–29 years). Due to unequal variances (decades), tissue density was logarithmically transformed to obtain equal variances.

Linear regression analyses were used to assess the correlation between mechanical and physical properties. The linearity of the properties in relation to age was first checked. If they showed nonlinear relationships with age, then polynomial regression (second degree fit) was used to describe the variations. Stepwise multiple linear regression analyses were used to assess the correlations between one mechanical property (Young's modulus, ultimate stress, ultimate strain or failure energy) as dependent variable and seven physical properties (tissue, collagen, apparent and apparent ash densities, collagen and mineral concentrations, volume fraction) as independent variables. The paired t-test was used to compare the properties between the condyles.

Study II

All the statistic analyses (SPSS software) were based on the entire data sets as the methods described in Study I. The paired t-test was first performed to compare the properties between the medial and lateral condyles. Linear regression analyses were further used to assess the association between the properties overall, and the medial or the lateral condyle and age. Then the slopes and intercepts of the linear regressions were tested to assess whether there were significant differences in age-related trends in the microstructural properties between the condyles. Linear and multiple regression analyses were used to assess the associations between Young's modulus and microstructural properties. The data were then divided into young (16–39 years), middle (40–59 years) and old age groups (60–85 years). One-way ANOVA was used to compare the properties in the three different age groups.

Study III

The data were divided into young (16–39 years), middle (40–59 years) and old age groups (60–83 years). The analyses were based on the same methods as in study I.

Study IV

A T-test, based on the mean values for each condyle (each condyle represented by one set of values), was used to compare these properties between groups. Due to non-normal data but equal variance, the Wilcoxon Matched Pairs Signed-Rank Test was applied to compare the stiffnesses of cartilage between groups. Linear regression analysis was conducted to assess the association of different properties between groups.

For all statistical analyses, a p-value <0.05 was considered significant.

Results

4.1 Mechanical properties

4.1.1 Age-related variations in the mechanical properties of trabecular bone

Analyses of age-related variations in the mechanical properties in the three age groups (young, middle and old) are summarized in Table I (based on ANOVA).

The Young's modulus (Figure 7) showed an initial increase but no significant difference between young and middle age groups. The Young's modulus was significantly larger in the middle age group than in the old age group ($p<0.001$), with a maximum at 40–50 years, and declined significantly after 60 years ($p<0.001$).

Ultimate stress showed no significant difference between the young and the middle age groups, and was lowest in the old age group ($p<0.001$). Comparison of data in different decades showed that this value was constant between 30 and 59 years ($p=0.76$), and declined after 60 years ($p<0.001$).

Ultimate strain in the young age group proved to be significantly larger than in the old age group ($p<0.05$), with no difference between young and middle age groups. Comparison of data in different decades showed that this value was maximal in the younger age group (16–29, $p<0.05$), and did not vary significantly in the other age groups (30–83 years, $p=0.17$).

Failure energy was lowest in the old age group ($p<0.001$) and showed no significant difference between young and middle age groups. However, comparison of data in different decades showed that this value was largest in the younger age group (16–29 years, $p<0.001$), constant between 30 and 59 years ($p=0.08$), and declined beyond 60 years ($p<0.001$).

4.1.2 Age-related variations in the mechanical properties of cartilage-bone complex

Analyses of age-related variations in the mechanical properties of cartilage and bone in the three age groups (young, middle and old) are summarized in Table II.

In relation to age, the Young's modulus of cartilage (Figure 8) showed an initial increase until 29 years, was found to be constant between 30 and 50, and showed a significant decline thereafter. ANOVA showed no significant difference between the young and the middle age groups. Young's moduli were significantly larger in the young and the middle age groups than in the old age group ($p<0.05$), with a maximum at 40 years, and declined significantly after 50 years ($p<0.001$).

Table I. Age-related variations [mean (SD)] in the mechanical & physical/compositional properties

Measurement	Young	Middle	Old	p-value
Mechanical				
Young's modulus (MPa)	654 (304)	829 (422)	613 (319)	<0.001
Ultimate stress (MPa)	10.6 (3.64)	9.86 (2.56)	7.27 (3.04)	<0.001
Ultimate strain (%)	2.48 (0.64)	2.12 (0.64)	2.05 (0.60)	<0.05
Failure energy (kJ/m^3)	145 (61)	112 (36)	87 (41)	<0.001
Physical/compositional				
Tissue density (g/cm^3)	2.21 (0.05)	2.18 (0.10)	2.20 (0.07)	0.88
Apparent density (g/cm^3)	0.52 (0.10)	0.51 (0.11)	0.40 (0.10)	<0.001
Mineral concentration (%)	66.4 (1.4)	66.6 (3.4)	66.5 (2.5)	0.87
Apparent ash density (g/cm^3)	0.34 (0.07)	0.34 (0.08)	0.27 (0.07)	<0.001
Collagen concentration (%)	25.7 (1.2)	24.9 (1.0)	25.3 (1.2)	0.06
Collagen density (g/cm^3)	0.13 (0.03)	0.13 (0.03)	0.10 (0.03)	<0.001
Volume fraction (%)	23.4 (4.6)	23.4 (5.7)	18.4 (5.0)	<0.001

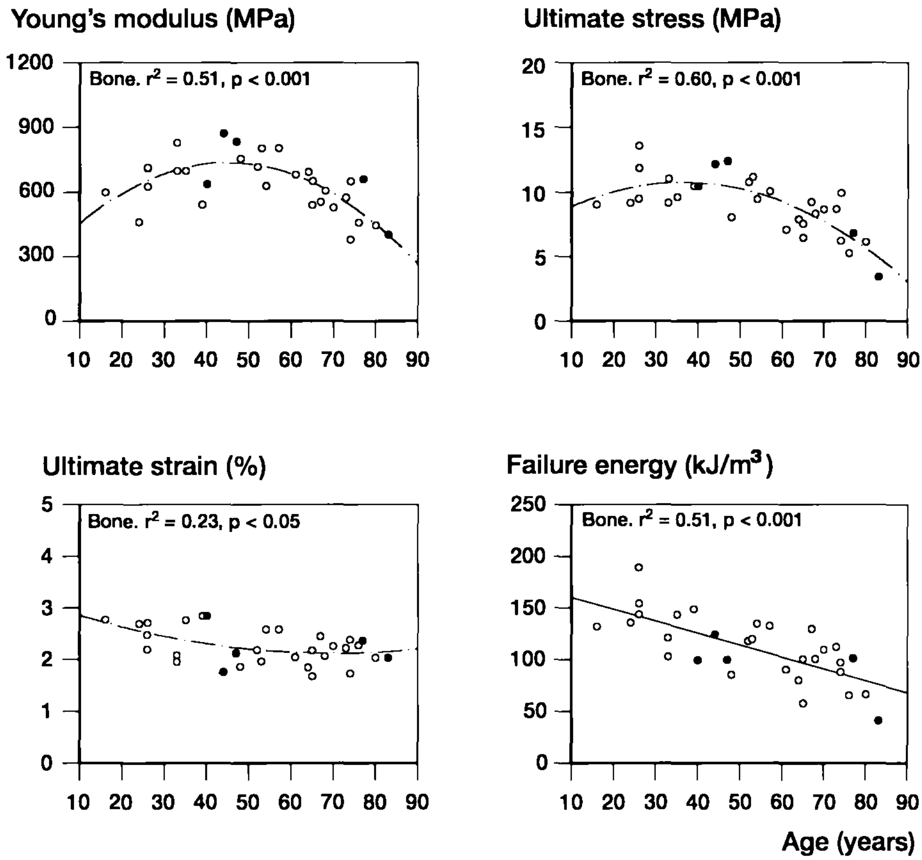

Figure 7. Age-related variations in the mechanical properties of human tibial trabecular bone, using the mean value of each knee. Due to the non-linear relationship of Young's modulus, ultimate stress, and ultimate strain with age, a second-degree polynomial fit (dash line) was used (filled circle = female (n_1=5), open circle = male (n_2=26)).

Table II. Analyses of age-related variations in the mechanical properties of cartilage and bone

Mechanical parameter	Mean (Level separation: age group)			
	Young	Middle	Old	p-value
Cartilage				
Young's modulus (MPa)	122 (b)	123 (b)	76 (a)	<0.01
Elastic energy (kJ/m^3)	141 (b)	124 (b)	83 (a)	<0.001
Viscoelastic energy (kJ/m^3)	42.5 (b)	35.7 (b)	22.9 (a)	<0.001
Relative energy loss (-)	0.24 (-)	0.22 (-)	0.22 (-)	0.87
Bone				
Young's modulus (MPa)	1393 (b)	1287 (b)	939 (a)	<0.01
Elastic energy (kJ/m^3)	12.3 (b)	11.9 (b)	7.7 (a)	<0.001
Viscoelastic energy (kJ/m^3)	0.56 (b)	0.63 (b)	-0.08 (a)	<0.01
Relative energy loss (-)	0.04 (b)	0.05 (b)	-0.03 (a)	<0.02

Note: All the mechanical properties (except for relative energy loss) of cartilage and bone were analyzed based on ANOVA. Due to non-equal variance, relative energy loss of cartilage and bone were analyzed based on non-parametric test. Level separation: "b" greater than "a".

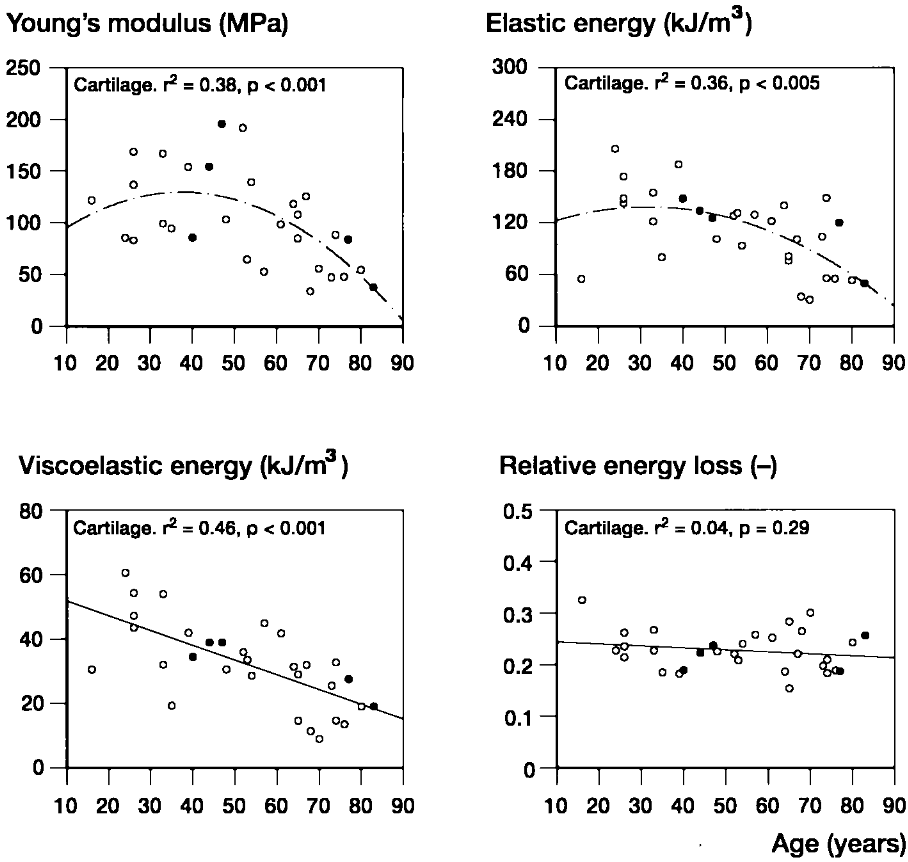

Figure 8. Age-related variations in the mechanical properties of human tibial articular cartilage (filled circle = female, n=5, open circle = male, n=26). Due to non-linear relationship of the normalized stiffness and elastic energy with age, polynomial regression (second-degree fit, dashed line) was used.

The elastic energy of cartilage showed a variation similar to that of the Young's modulus. The viscoelastic energy of cartilage showed a maximum in the younger age group (16–29 years) and no significant difference between the young and the middle age groups. These values in the young and the middle age groups were significantly larger than those in the old age group ($p<0.005$). The relative energy loss of cartilage showed no substantial difference between age groups.

Both the Young's modulus and elastic energy of bone showed maximal values around 40 years. ANOVA showed no significant difference between the young and the middle age groups. These values in the young and the middle age groups were significantly larger than those in the old age group ($p<0.01$).

The viscoelastic energy and relative energy loss of bone showed no significant difference between the young and the middle age groups. These values in the young and the middle age groups were significantly larger than those in the old age group ($p<0.02$).

4.1.3 Changes in the stiffness in early-stage osteoarthrosis

The average values of the stiffnesses of cartilage and bone in OA, lateral comparison, medial age-matched, and lateral age-matched groups are summarized in Table III.

The stiffness of OA cartilage was significantly lower than that of the medial age-matched cartilage (reduced by 29%, $p=0.04$), whereas the stiff-

Table III. The mean values and the relationships of the stiffnesses between cartilage and bone in the four groups: osteoarthrosis (n=9), lateral comparison (n=9), medial age-matched (n=10) and lateral age-matched (n=10). The determination coefficients (r^2) and p-values were derived from linear regression analysis between cartilage and bone stiffnesses within the same group

Stiffness (MPa)	Cartilage mean (range)	Bone mean (range)	Cartilage vs. Bone r^2	p-value
Osteoarthrosis	58 (15–180)	237 (53–646)	0.07	0.51
Lateral comparison	61 (11–221)	218 (23–608)	0.57	0.02
Medial age-matched	82 (34–235)	311 (53–887)	0.63	0.006
Lateral age-matched	67 (16–170)	292 (30–723)	0.49	0.02

ness of OA cartilage did not differ significantly from that of the lateral comparison cartilage (p=0.76). Likewise, the stiffness of OA bone was 24% lower than that of the medial age-matched bone, however, due to large variation in data, this difference was not statistically significant (p=0.4).

The mean thickness of OA cartilage at the test location was 2.3 (1.8-3.0) mm which was significantly thinner than the lateral comparison cartilage at the test location [2.8 (1.8–4.2) mm], p=0.03. OA cartilage was also thinner than the medial age-matched cartilage at the test location [2.5 (1.7–3.6) mm], p=0.26. This reduction in thickness, however, was not correlated with the reduction in stiffness for OA cartilage, and no correlation was found between the thickness and the cartilage stiffness in the other three control groups.

The stiffness of OA cartilage was not correlated with bone stiffness (p=0.51), whereas the stiffness of the medial age-matched cartilage correlated significantly with bone stiffness (p=0.006). The significant correlations between cartilage and bone were also found in the lateral comparison group and the lateral age-matched group (Table III).

4.2 Physical/compositional properties

4.2.1 Age-related variations in tissue, apparent, apparent ash, and collagen densities

Tissue density (Figure 9) showed no significant variation with age (p=0.88). Comparison of data in different decades showed that this value was very constant (p=0.53).

Apparent density was lowest in the old age group (p<0.001), while no significant difference between young and middle age groups was found. Comparison of data in different decades showed that apparent density was constant until the age of 59 years (16–59, p=0.87), followed by a steady decline after 60 years (p<0.001).

Apparent ash density showed the same tendency as apparent density. These two parameters were strongly correlated (r^2=0.97, p<0.001) in accordance with the small variation of the tissue density. Volume fraction showed a tendency similar to apparent density, and it also correlated strongly with apparent density (r^2=0.96, p<0.001), and apparent ash density (r^2=0.90, p<0.001).

Collagen density was lowest in the old age group (p<0.001), whereas no significant difference between young and middle age groups was found. Comparison of data in different decades showed that this value was highest in the younger age group (16–29 years, p<0.001), constant between 30–59 years (p=0.65), followed by a significant decline beyond 60 years (p<0.001). The existence of a maximal value of collagen density in the younger age group (16–29 years) was different from what was found for apparent density, apparent ash density and volume fraction, although collagen density showed strong correlations with these parameters (r^2=0.94, r^2=0.88, r^2=0.90, p<0.001).

4.2.2 Age-related variations in collagen and mineral concentrations

Collagen concentration (Figure 9) showed no significant difference among the three age groups.

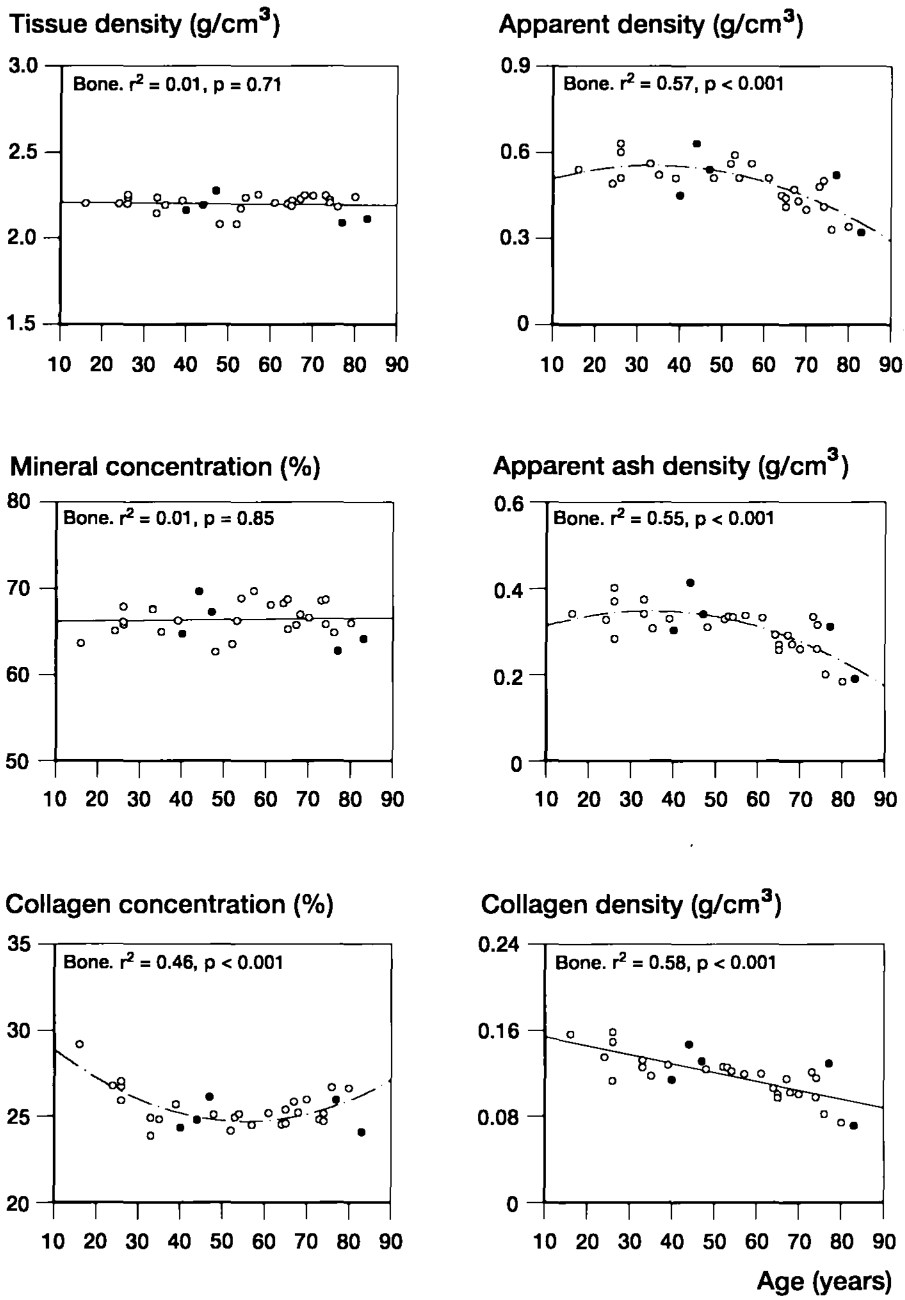

Figure 9. Age-related variations in the physical/compositional properties of human tibial trabecular bone, using the mean value of each knee. Due to non-linear relationship of collagen concentration, apparent density and apparent ash density with age, a second-degree polynomial fit (dashed line) was used (filled circle = female (n_1=5), open circle = male (n_2=26)).

However, comparision of data in different decades showed that collagen concentration was highest in the younger age group (16–29 years, $p<0.005$), but was constant thereafter throughout life (30-83 years, $p=0.56$).

Mineral concentration showed no significant variation with age ($p=0.87$). Comparison of data in different decades showed that this value, like tissue density, was very constant ($p=0.81$).

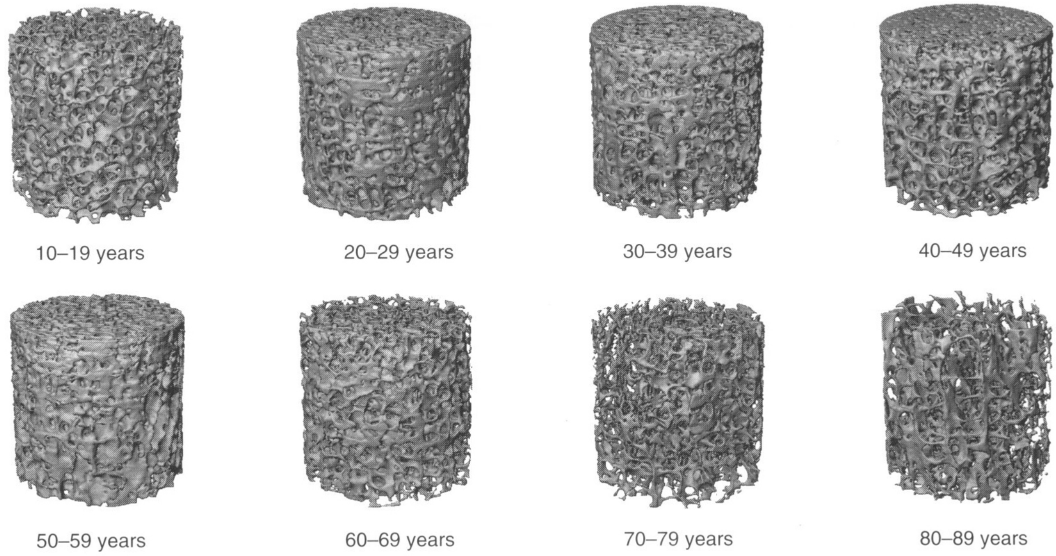

Figure 10. Three-dimensional reconstruction of cylindrical human tibial trabecular bone specimens from micro-CT images in each decade. Significant decline in density (volume fraction) and thinning in trabeculae after 60 years of age and a change in microstructure of trabecular bone from plate-like to more or less rod-like are seen. While these changes are not apparent between 20 and 59 years.

4.3 Structural properties

Typical 3-D reconstructions of cylindrical human tibial trabecular bone specimens from micro-CT images in each decade are shown in Figure 10. The average values of the structural properties of the tibiae as well as the values for the medial and lateral condyles and their differences are summarised in Table IV.

Table IV. Structural properties overall, and for the medial and lateral tibial condyles

Structural properties	Overall median (range) 95% CI of mean	Medial condyle median (range) 95% CI of mean	Lateral condyle median (range) 95% CI of mean	M. – L. mean ± SD	p–value (M. v. L.)
DA	5.44 (1.35–62) 6.22–8.38	5.07 (1.35–62) 5.48–9.21	5.86 (1.61–25.6) 6.12–8.38	0.09 ± 8.5	0.92
CD	6.86 (3.2–12.1) 7.24–7.94	6.86 (3.2–12.1) 6.75–7.64	7.37 (4.1–15.3) 7.45–8.52	–0.79 ± 2.36	<0.005
Vv	0.23 (0.10–0.39) 0.23–0.25	0.27 (0.10–0.39) 0.25–0.28	0.22 (0.10–0.39) 0.21–0.23	0.04 ± 0.07	<0.001
MTV	31.2 (13.4–69.1) 31.7–35.6	38.5 (13.9–69.1) 35.7–41.5	27.8 (13.4–59.4) 26.7–30.8	9.9 ± 14.3	<0.001
MMSV	104 (41.6–249) 103–115	107 (58.2–249) 103–120	103 (41.6–215) 99.2–115	4.22 ± 33.4	0.26
BS/BV	17.9 (8.3–35.6) 18.1–19.8	16.2 (8.33–35.6) 15.9–18.0	20.3 (10.5–35.0) 19.8–22.2	–4.0 ± 5.2	<0.001
BS/TV	3.95 (2.09–5.42) 3.81–3.99	4.00 (2.38–4.92) 3.85–4.08	3.89 (2.09–5.42) 3.70–3.98	0.12 ± 0.56	0.06

Note: DA = The degree of anisotropy (–), CD = Connectivity density (mm^{-3}), Vv = Volume fraction (–), MTV = Mean trabecular volume (mm^3), MMSV = Mean marrow space volume (mm^3), BS/BV = Bone surface–to–volume ratio (mm^{-1}), BS/TV = Bone surface density (mm^{-1}), and CI = confidence interval.

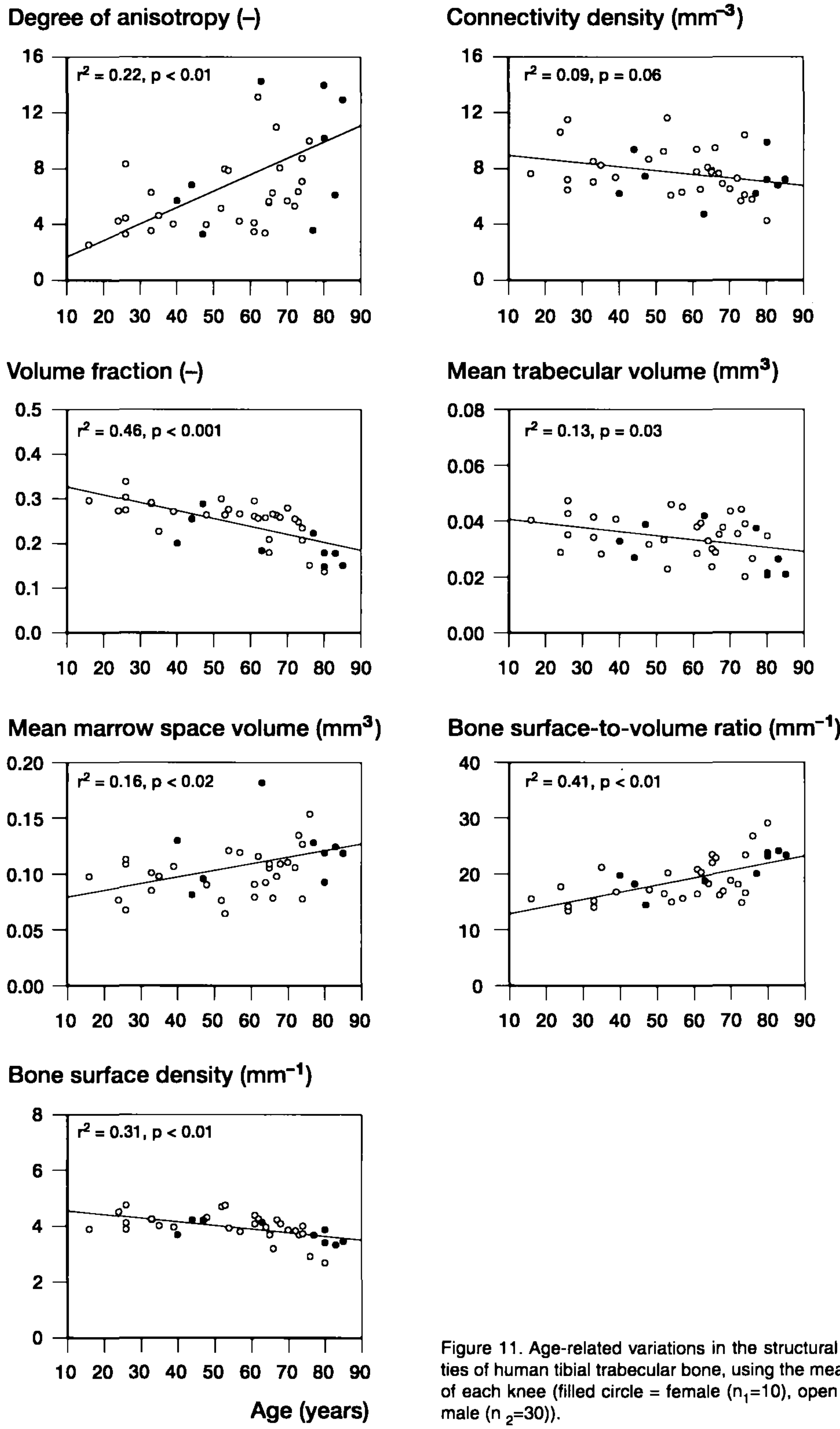

Figure 11. Age-related variations in the structural properties of human tibial trabecular bone, using the mean value of each knee (filled circle = female (n_1=10), open circle = male (n_2=30)).

Table V. Expressions of degree of anisotropy overall, and for the medial and lateral tibial condyles

Degree of anisotropy	Overall median (range) 95% CI of mean	Medial condyle median (range) 95% CI of mean	Lateral condyle median (range) 95% CI of mean	M. – L. mean ± SD	p–value (M. v. L.)
Primary–to–secondary	2.96 (1.0–17.2) 3.62–4.55	2.96 (1.06–17.2) 3.34–4.74	2.96 (1.0–15.2) 3.50–4.76	–0.09 ± 3.74	0.84
Secondary–to–tertiary	1.48 (1.0–15.8) 1.64–2.10	1.46 (1.07–6.24) 1.54–1.88	1.51 (1.03–15.8) 1.60–2.45	–0.32 ± 1.78	0.11
Primary–to–tertiary	5.44 (1.35–62) 6.22–8.38	5.07 (1.35–62.7) 5.48–9.21	5.86 (1.61–25.6) 6.12–8.38	0.09 ± 8.50	0.92

4.3.1 Age-related variations in architectural anisotropy

The degree of anisotropy (Figure 11) was highest in the old age group ($p<0.03$), with no difference between young and middle age groups ($p=0.14$). Analysis of data in decades showed that it was constant until 79 years ($p=0.48$), followed by a significant increase ($p<0.03$). Expression the degree of anisotropy strengths of three main directions: primary-to-secondary, secondary-to-tertiary, and primary-to-tertiary overall, the medial and the lateral condyles are summarized in Table V. All specimens in this study showed anisotropic, i.e. the ratios of three directions differ significantly from 1 ($p<0.01$, Table V). There does not seem to be transversely isotropic as evidenced by the fact that secondary-to-tertiary ratio differs significantly from 1 ($p<0.01$). The anisotropy strength distributions showed a similar pattern, i.e. the anisotropies were primary direction-orientated (Figure 12).

4.3.2 Age-related variations in connectivity, bone volume fraction, mean trabecular volume and mean marrow space volume

Connectivity density (Figure 11) of human tibial cancellous bone did not vary significantly among young, middle and old age groups ($p=0.22$), nor among decade groups ($p=0.68$).

Volume fraction, on the other hand, was lowest in the old age group ($p<0.001$), with no difference between young and middle age groups ($p=0.18$). The mean trabecular volume showed no difference among young, middle and old age groups ($p=0.15$), nor among decade groups ($p=0.07$). The mean marrow space volume showed no difference among young, middle and old age groups ($p=0.06$), nor among decade groups ($p=0.09$).

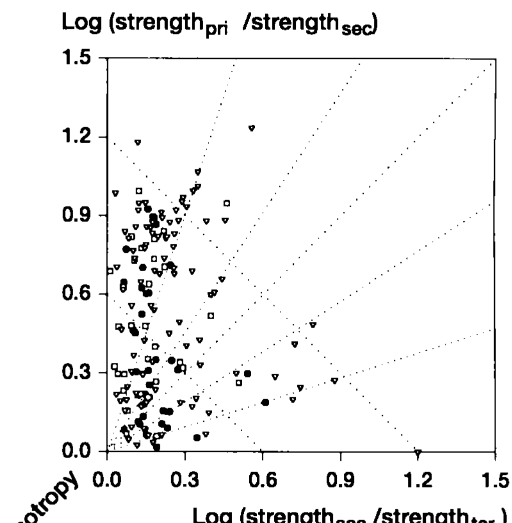

Figure 12. The distributions of anisotropy is shown in a so-called "two-axis ratio plot" (Fisher et al., 1987) by plotting the logarithm to the ratios of the anisotropy strengths (τ) in the material symmetry directions against each other. Points at the origin have isotropic strength ($\tau_1 = \tau_2 = \tau_3$), whereas points along the abscissa have one low and two high strengths ($\tau_1 < \tau_2 = \tau_3$), and points along the ordinate have one high and two low strengths ($\tau_1 = \tau_2 < \tau_3$). Points at the same diagonal (y + x = constant) will have the same anisotropy ratio: log (τ_3 / τ_2) + log (τ_2 / τ_1) = log (τ_3 / τ_1) = log (anisotropy ratio) (Kabel et al., 1999). This figure showed that the anisotropy strength distributions had a similar pattern, i.e. the anisotropy strengths were primary direction-orientated.

Table VI. Linear regression analysis between physical/compositional parameters and mechanical properties

	Young's modulus		Ultimate stress		Ultimate strain		Failure energy	
	r^2	p-value	r^2	p-value	r^2	p-value	r^2	p-value
Tissue density	0.002	0.82	0.02	0.41	0.07	0.15	0.04	0.19
Apparent density	0.31	<0.001	0.71	<0.001	0.02	0.44	0.47	<0.001
Mineral concentration	0.09	0.09	0.03	0.34	0.005	0.70	0.01	0.58
Apparent ash density	0.35	<0.001	0.72	<0.001	0.01	0.53	0.46	<0.001
Collagen concentration	0.08	0.13	0.0001	0.99	0.25	<0.005	0.08	0.13
Collagen density	0.22	<0.05	0.66,	<0.001	0.07	0.15	0.54	<0.001
Volume fraction	0.28	<0.005	0.65	<0.001	0.007	0.64	0.40	<0.001

4.3.3 Age-related variations in bone surface-to-volume ratio and bone surface density

Bone surface-to-volume ratio was highest in the old age group ($p<0.001$), with no difference between young and middle age groups ($p=0.24$). Comparison of data in different decades showed that it was constant until 59 years ($p=0.44$), followed by a steady decrease ($p<0.001$).

Bone surface density was lowest in the old age group ($p<0.005$), with no difference between young and middle age groups ($p=0.89$). Comparison of data in different decades showed that it was constant until 69 years ($p=0.55$), followed by a steady decrease ($p<0.003$).

4.4 Relationships between various properties

4.4.1 Relationships between physical/compositional and mechanical properties of bone

The relationships between physical/compositional parameters and mechanical properties are based on linear regression analysis, and summarized in Table VI. Stepwise multiple linear regression analysis showed that apparent ash density showed the best correlation with Young's modulus. Apparent ash density and apparent density showed the best correlation with ultimate stress. Of all parameters only collagen concentration correlated with ultimate strain. Collagen density correlated best with failure energy, whereas apparent density and apparent ash density showed the second best correlation with failure energy.

4.4.2 Relationships between mechanical properties of cartilage and bone

Linear regression analysis showed significant correlations between the Young's moduli of cartilage and bone and between the elastic energies of cartilage and bone ($r^2=0.34$ and $r^2=0.65$, $P<0.001$). The Young's modulus of bone was 11 times higher, and the elastic energy of bone was 11 times lower than the respective properties of cartilage ($p<0.001$).

The mean thickness of the cartilage at the test location was 2.10 ± 0.43 mm. Its thickness at the lateral condyle was 30% higher than that at the medial condyle (medial: 1.82 ± 0.26, lateral: 2.36 ± 0.39 mm, $p<0.001$). From the young age group (16-29 years) to the old age group (over 80 years), the mean thickness of cartilage at the test location decreased by 12%, $p=0.39$ (medial decreased by 7%, $p=0.38$, lateral decreased by 13%, $p=0.37$). Linear regression analysis showed that the mean cartilage thickness correlated significantly with age ($r^2=0.21$, $p<0.02$). However, only the lateral cartilage thickness declined significantly with age ($r^2=0.29$, $p<0.002$), whereas the medial cartilage thickness did not vary with age ($r^2=0.14$, $p=0.16$).

4.5 Differences between medial and lateral condyles

4.5.1 Mechanical, physical/compositional properties of normal trabecular bone

The average values of the mechanical and physical properties of the specimens as well as the values for the medial and lateral condyles are shown in Table VII. The average values of Young's mod-

Table VII. Mechanical and physical/compositional properties of tibial trabecular bone

Measurement	Medial condyle mean (SD)	Lateral condyle mean (SD)	Med – Lat mean (SD)	p-values (M vs L)
Mechanical				
Young's modulus (MPa)	708 (402)	560 (352)	144 (225)	<0.001
Ultimate stress (MPa)	9.34 (3.67)	8.22 (3.21)	1.58 (2.53)	<0.03
Ultimate strain (%)	2.21 (0.69)	2.24 (0.66)	0.26 (1.33)	0.76
Failure energy (kJ/m^3)	115 (55)	103 (48)	14.6 (37.7)	0.12
Physical/compositional				
Tissue density (g/cm^3)	2.19 (0.07)	2.21 (0.07)	-0.02 (0.06)	0.09
Apparent density (g/cm^3)	0.50 (0.11)	0.42 (0.11)	0.08 (0.08)	<0.001
Mineral concentration (%)	66.0 (2.5)	66.8 (2.5)	-0.78 (2.07)	0.10
Apparent ash density (g/cm^3)	0.33 (0.07)	0.28 (0.07)	0.05 (0.05)	<0.001
Collagen concentration (%)	25.4 (1.4)	25.5 (1.3)	-0.10 (1.29)	0.75
Collagen density (g/cm^3)	0.13 (0.03)	0.11 (0.03)	0.02 (0.02)	<0.001
Volume fraction (%)	23.0 (5.2)	19.2 (5.3)	3.79 (4.26)	<0.001

Table VIII. Structural properties (mean ± SD) of the medial and the lateral condyles of human tibial trabecular bone

Measurement	Medial condyle	Lateral condyle	Med. – Lat.	p-value (M. *vs.* L.)
Degree of anisotropy (-)	7.35 ± 8.38	7.25 ± 5.09	0.09 ± 8.5	0.93
Connectivity density (mm^{-3})	7.19 ± 1.99	7.99 ± 2.41	-0.79 ± 2.36	<0.005
Volume fraction (%)	0.26 ± 0.07	0.22 ± 0.06	0.04 ± 0.07	<0.001
Mean trabecular volume (mm^3)	38.6 ± 1.3	28.7 ± 0.9	9.9 ± 14.3	<0.001
Mean marrow space volume (mm^3)	111.2 ± 37.3	107.0 ± 35.0	4.2 ± 33.4	0.46
Surface-to-volume ratio (mm^{-1})	16.9 ± 4.7	21.0 ± 5.4	-4.0 ± 5.2	<0.001
Surface density (mm^{-1})	3.96 ± 0.53	3.84 ± 0.63	0.12 ± 0.56	0.06

ulus, ultimate stress, apparent density, collagen density, apparent ash density and volume fraction from the medial condyle were significantly larger (14–26%) than those from the lateral condyle ($p<0.05$-$p<0.001$). The ultimate strain, failure energy, tissue density, mineral concentration and collagen concentration showed no significant difference between the condyles.

4.5.2 Structural properties of normal trabecular bone

The average values of the structural properties of the specimens as well as the values for the medial and lateral condyles and their differences are summarised in Table VIII. The average values of volume fraction and mean trabecular volume from the medial condyle were significantly larger than those from the lateral condyle (18% and 32%, respectively, $p<0.001$). The connectivity density and bone surface-to-volume ratio from the medial condyle were significantly lower than those from the lateral condyle (6% and 20%, respectively, $p<0.005$ and $p<0.001$). There was no significant difference in the degree of anisotropy, mean marrow space volume, bone surface, and bone surface density between the condyles. None of the slopes or intercepts of the microstructural properties vs. age differed significantly between medial and lateral condyles (all $p>0.10$). These results showed that all the microstructural properties from the medial and lateral condyles had the same age-related trends.

Discussion

5.1 Limitation and advantage of the methods used

The well established compressive testing methods have provided a powerful tool for the evaluation of trabecular bone mechanical properties (Linde et al., 1988; Linde et al., 1989; Linde, 1994). By using the novel combined testing technique (Røhl et al., 1997), it has been possible to investigate the mechanical properties of the cartilage-bone complex simultaneously. Based on the standardized methods, various physical/compositional properties can be measured with accuracy and precision (Danielsen and Andreassen, 1988; Ding et al., 1997; Grant, 1965; Neuman and Logan, 1950; Sharp et al., 1990). Recent development of 3-D imaging techniques of trabecular bone has made 3-D quantification of trabecular microstructure possible in a true, unbiased and assumption-free manner (Hildebrand and Rüegsegger, 1997a; Hildebrand and Rüegsegger, 1997b; Odgaard et al., 1990; Odgaard and Gundersen, 1993; Odgaard et al., 1997).

1) Quantification of mechanical properties

The use of conventional mechanical testing, such as compression or tensile testing, can easily acquire knowledge of the mechanical properties of trabecular bone. Although a simple approach, numerous inherent problems exist by using these testing techniques. The major problem is end-plate error, which accounts for a systematic underestimation of the Young's moduli by 20–40% (Linde, 1994; Odgaard and Linde, 1991) and a random error of about 12.5% (Keaveny et al., 1997). Other problems include unknown friction between the end platens (Linde and Hvid, 1989), specimen geometry (Keaveny and Hayes, 1993; Linde et al., 1992), structural end phenomena (Odgaard et al., 1989; Zhu et al., 1994), continuum assumption (Harrigan et al., 1988), storage (Linde and Sorensen, 1993), effect of drying (Carter and Hayes, 1977; Linde, 1994), and effect of temperature (Linde, 1994).

Trabecular bone and cartilage have both been demonstrated to be a structure rather than a material, which makes it impossible to remove small samples for testing without disrupting its structural integrity. One must realize that mechanical testing in vivo is impossible, and none of the mechanical testings in vitro are perfect, although it has been argued that the effects of artifacts can be reduced by an improved mechanical testing technique (Keaveny et al., 1994a). A large-scale finite element model that simulates mechanical testing has provided some hope for this issue (van Rietbergen et al., 1995a).

Despite of these, the reproducibilities of both mechanical testings of cartilage and bone are excellent. Therefore, studies based on the same methods are comparable. In general, these well established compressive testing methods have provided a strong tool for the evaluation of trabecular bone mechanical properties (Linde et al., 1988; Linde and Hvid, 1989; Linde et al., 1989; Linde, 1994). By using the novel combined testing technique (Røhl et al., 1997), it has been possible to investigate the mechanical properties of cartilage-bone complex simultaneously, and to reveal changes in the mechanical properties under pathological conditions.

2) Quantification of physical/compositional properties

Based on the standardized methods, various physical/compositional properties can be measured with accuracy and precision (Danielsen and Andreassen, 1988; Ding et al., 1997; Grant, 1965; Neuman and Logan, 1950; Sharp et al., 1990). Physical properties, such as collagen density, apparent density and volume fraction are considered to be the major determinants of mechanical properties (Ding et al., 1997; Goldstein et al., 1993; Odgaard et al., 1997). Compositional properties, such as collagen and mineral concentrations, provide additional information to the changes in mechanical properties (Ding et al., 1997).

3) Quantification of trabecular bone microstructure

Recent developments in 3-D imaging technique has made true 3-D quantification of cancellous bone microstructure possible. Structural parameters, such as architectural anisotropy, connectivity, structure model type, and trabecular thickness can be calculated directly from 3-D images. These methods, which are unbiased and free of assumptions, enable a detailed and versatile quantification of 3-D architecture (Hildebrand and Rüegsegger, 1997a; Hildebrand and Rüegsegger, 1997b; Odgaard et al., 1990; Odgaard and Gundersen, 1993; Odgaard et al., 1997), and a better understanding of trabecular microstructure and its relationships with mechanical properties.

5.2 Age variations in the properties of trabecular bone

5.2.1 Age variations in the mechanical properties of trabecular bone

Age-related decline in the mechanical properties of human trabecular bone primarily occurs after 50–60 years of age. However, different trends exist for different anatomical locations. For the human tibia, Young's modulus, ultimate stress, and failure energy increase with age to reach maximal values at 30–50 years of age, and decline only after 60 years of age (Ding et al., 1997). Ultimate strain is higher in the younger age (16–29 years), and remains relatively constant thereafter with increasing age. For human vertebra, the mechanical properties have a maximum in the younger age and follow a linear decline with age (McCalden et al., 1997; Mosekilde et al., 1987). A significant age-related decline occurs in ultimate stress (Lindahl, 1976; Mosekilde et al., 1987; Weaver and Chalmers, 1966), Young's modulus (Lindahl, 1976; Mosekilde et al., 1987), and failure energy (Mosekilde et al., 1987). It has been observed that, from 20–80 years of age, a linear decrease of 75–80% occurs by vertical compression, and 90–96% by horizontal compression for ultimate stress, ultimate stiffness and failure energy of vertebral trabecular bone (Mosekilde et al., 1987). For human femoral trabecular bone, the compressive strength declines linearly by 8.5% per decade (McCalden et al., 1997).

It is interesting information that failure energy shows a maximum between 16 and 29 years for human tibial trabecular bone. This suggests that tibial trabecular bone is tougher in the younger age (Ding et al., 1997), i.e. fracture requires more energy. This assumption is supported by a maximum in ultimate strain in the younger age group, and is also in accordance with a similar finding in cortical bone (Currey and Butler, 1975).

Mechanical properties of trabecular bone vary significantly between anatomic locations. For human tibial trabecular bone, Young's moduli of fresh frozen specimens are mostly in a range from 200 to 900 MPa, ultimate stress 5.3–9.5 MPa, ultimate strain 2–2.7%, and failure energy 30–300 kJ/m^3 (Ding et al., 1997; Hvid et al., 1983; Linde, 1994). For human vertebral trabecular bone, Young's moduli of fresh frozen specimens are mostly in a range of 25–250 MPa, ultimate stress 2–4 MPa, ultimate strain 2–2.7% MPa, and failure energy 20–300 kJ/m^3 (Goldstein, 1987; Linde, 1994; Mosekilde et al., 1987). For human proximal femoral trabecular bone, Young's moduli of fresh frozen specimens are mostly in a range of 58–2248 MPa, ultimate stress 0.45–16.2 MPa, and failure energy 21–238 kJ/m^3 (Ciarelli et al., 1991). For human distal femoral trabecular bone, Young's moduli of fresh frozen specimens are mostly in a range of 58–2942 MPa, and ultimate stress 0.56–66 MPa (Ciarelli et al., 1991; Goldstein, 1987).

Huge variations in the mechanical properties have been observed. These may be the results of different protocols in specimen preparation (e.g. fresh frozen versus dry defatted) (Carter and Hayes, 1977; Lindahl, 1976), storage and freezing (Linde and Sorensen, 1993; Panjabi et al., 1985), specimen geometry (Carter and Hayes, 1977; Linde et al., 1992), temperature (Linde, 1994), testing direction (Turner and Cowin, 1988), and preconditioning before actual mechanical testing (Hvid et al., 1989). Data would not be comparable, were it not subject to similar testing condition.

Strain rate has been shown to be a good stimulus for bone surface remodeling (Goldstein et al., 1995; Luo et al., 1995). This is probably especially true for the tibia, since tibial trabecular bone strain at a given load is fairly constant throughout life, although higher in the younger age (Ding et al., 1997). Thus, mechanical strain of trabecular bone performs a significant role in triggering bone remodeling. Interestingly, ultimate strain correlated significantly with collagen concentration, the major organic component of trabecular bone, but not the densities. This finding might indicate that the organic component of trabecular bone rather than the non-organic component (mineral) influenced mechanical strain.

For cortical bone, age-related variations in mechanical properties have also been reported (Currey and Butler, 1975; Currey, 1979; McCalden et al., 1993). McCalden et al. (McCalden et al., 1993) found that ultimate stress, ultimate strain and failure energy of cortical bone decreased linearly by 5, 9, and 12 percent per decade. It is of interest to note that, although ultimate stress and failure energy from both cortical and trabecular bone decreased with aging, ultimate strain for cortical bone also decreased significantly with aging, whereas for tibial trabecular bone it showed a maximum in the younger age group and was constant thereafter (Ding et al., 1997).

Young's modulus of tibial trabecular bone varies significantly with age (Ding et al., 1997). This observation differs from that of cortical bone as reported by McCalden et al. (McCalden et al., 1993), who found that the modulus of cortical bone changed little with age. This observation is in agreement with Currey and Butler (Currey and Butler, 1975), who also showed that the elastic modulus and bending strength both increased with age until about 30 years of age, and decreased thereafter.

Previous studies (McCalden et al., 1993) showed the importance of age-related changes in porosity for the decline in mechanical properties. Porosity accounted for 76% of the reduction in the ultimate stress of cortical bone. This may be compared with trabecular bone volume fraction (1-porosity), that has a strong effect on the mechanical properties of trabecular bone (Ding et al., 1997).

5.2.2 Age variations in the physical/compositional properties of trabecular bone

Bone density has been most intensively investigated. It has been well documented that density decline occurs with increasing age in both sexes, and is accelerated in females after menopause at the age of approximately 50–60 years. Density plays a very important role in the determination of mechanical strength of both trabecular bone and cortical bone. Apparent density, apparent ash density, volume fraction, and collagen density decline with age for trabecular bone. However, tissue density does not change with age (Ding et al., 1997).

The literature value for tissue density of human trabecular bone is approximately 1.9–2.2 g/cm^3 (Ding et al., 1997; Gong et al., 1964), and for cortical bone approximately 2.0 g/cm^3 (Gong et al., 1964). Different values of tissue density may be due to the different methods applied for the determination of dry weight, such as freeze-drying, centrifugation, or direct evaporation at room temperature. It seems that freeze-drying and centrifugation methods are preferable, both give consistent results (Ding et al., 1997; Sharp et al., 1990).

A strong correlation was found among collagen density, apparent ash density, apparent density and volume fraction (r^2=0.88–0.94) (Ding et al., 1997). Therefore, any one of these parameters will carry almost identical information. These parameters all had parallel declines after 50–60 years of age, with an exception of a maximum of collagen density in younger age (Ding et al., 1997). It has been found that, despite the bone substance lost (density decline), the substance itself remains constant in composition as evidenced by the finding of constant tissue density, mineral concentration and collagen concentration (only maximal in younger ages) throughout adult life (Ding et al., 1997). It is an important finding that trabecular bone composition was constant throughout life. Thus, the decrease in mechanical properties seems to be mainly a consequence of loss of bone substance (quantity), rather than a decrease in the quality of bone substance itself (Ding et al., 1997; McCalden et al., 1997). This result is in agreement with the finding in cortical bone (McCalden et al., 1993).

The bone matrix consists of approximate 90% collagen and 10% non-collagenous organic components including proteoglycans. Proteoglycans have been shown to have some influence on mechanical properties of bone tissue. Decorin, a small proteoglycan present in bone is known to be intimately associated with collagen (Scott, 1988; Uldbjerg and Danielsen, 1988). The intimate relationship exists between proteoglycans and collagen in bone tissue by changed proteoglycan orientation following mechanical loading (Skerry et al., 1990). Proteoglycans bind interstitial fluid especially known to influence the mechanical properties of cartilage (Armstrong and Mow, 1982). Bound water in organic bone matrix could also influence the mechanical properties of bone. Thus, variation in proteoglycan composition and quantity in the bone specimens tested might contribute to the variation in mechanical parameters. Further investigation is needed to elucidate this contribution.

Collagen density was found to be a better predictor than mineralization and density in determining failure energy of trabecular bone. Collagen density alone could explain 54% of the variation of failure energy (Ding et al., 1997). This result might indicate that failure energy depends largely on the organic component, rather than on mineral.

5.2.3 Age variations in the structural properties of trabecular bone

The most striking feature of trabecular bone is perhaps its architectural anisotropy, which has been quantified by different methods. The most common and well-known method is the mean intercept length (MIL). Using this method, the degree of anisotropy has been reported to be 1.62 for human trabecular bone (Goulet et al., 1994), 1.73 for bovine trabecular bone and 1.54 for human proximal tibial trabecular bone (Turner et al., 1990), and 1.55 for sperm whale trabecular bone (Odgaard et al., 1997). Since the MIL method can not explain all features of trabecular architecture, the volume based methods, such as star volume distribution (SVD), star length distribution (SLD), and volume orientation (VO) have been introduced (Odgaard, 1997). Using SVD method, degree of anisotropy has been reported to be 7.30 (range 1.35–62.07) for human tibial trabecular bone, and 10.25 (range 2.20–16.97) for sperm whale trabecular bone (Odgaard et al., 1997). It has been observed that a large variation of degree of anisotropy exists in trabecular bone investigated. Direct comparison with other studies may be difficult due to differences in methods and species. Nevertheless, the SVD, out of all architectural methods, seems best to predict the mechanical anisotropy direction (Odgaard et al., 1997).

It is an interesting finding that the degree of anisotropy for human tibial trabecular bone increases significantly with age occurring first after 80 years of age (Ding et al., 2000). This phenomenon can best be interpreted as a consequence of aging and the decline in bone mass. Thus, the aging trabeculae seem to align more strongly to the primary direction. This main direction is parallel to the longitudinal loading axis of the tibia (Figure 10). These findings suggest that cancellous bone architecture may re-organize continually to adapt to the mechanical environment during aging. This is evidenced by the constant nature of connectivity. i.e. the numbers of multiple connections in aging cancellous bone are unchanged, but the trabeculae orient strongly to the main direction. The observed increase of anisotropy in aging trabeculae may indicate a consequence of structural adaptation secondary to aging-induced bone loss. This phenomenon suggests that the microstructure of cancellous bone re-organizes continually to adapt to the mechanical loading environment in aging bone.

Connectivity density of normal tibial trabecular bone has been reported not to have a general relationship with age, however, a trend exists (Ding et al., 2000). Age-related variation in connectivity may depend on anatomical locations. It is possible that changes in connectivity with aging might happen in areas in the axial skeleton, where bone remodeling is most extensive (Mosekilde, 1998). Age-related variation in connectivity may also depend on gender. Investigation on iliac crest connectivity showed that connectivity for males was independent of age, whereas for females connectivity decreased significantly with age (Thomsen et al., 1998). This seems reasonable, as aging resulting in bone loss is more pronounced in female than in male, decline in connectivity may occur as a consequence of removal of entire trabecular elements, the main bone loss pattern for female (Parfitt, 1987). It will be of great interest to see the variation in connectivity in aging female and age-related diseases, such as osteoporosis and osteoarthrosis, and its influence on mechanical properties.

It has been proposed that under pathological conditions, such as osteoporosis, the decrease in connectivity density might occur as a consequence of the loss of trabecular bone elements (Parfitt, 1987). A few interesting studies have recently been published. One study, based on stereological technique in vitro, found that the reduction in the connectivity of vertebral trabecular bone is a long-term consequence of ovariectomy in rat (Boyce et al., 1995). Other studies, based on X-ray tomographic microscopy in vivo, showed that a significant rapid deterioration in trabecular connectivity was found in the ovariectomized rat (Kinney et al., 1995; Lane et al., 1998). It has been reported that a significant increase in connectivity and marrow star volume occurs in the vertebral trabecular bone of the calcium-restricted ovariectomized minipigs, which suggested that trabecular plates were transforming into rods by perforation (Boyce et al., 1995).

Bone surface to volume ratio for tibial trabecular bone increased significantly with age, whereas bone surface declined significantly with age (Ding et al., 2000; McCalden et al., 1997; Parfitt et al., 1983). Bone volume declined much more pronounced than bone surface. From the younger age group (16 to 29 years) to the group over 80 years, bone surface was reduced by 19%, whereas bone volume was reduced by 51%, 2.7 times as much (Figure 11) (Ding et al., 2000).

5.2.4 Properties of osteoarthrotic trabecular bone

For the past three decades, research into the etiology of OA has been concentrated on the articular cartilage destruction where damages were clearly visible. OA develops and changes very slowly, making it very difficult to follow over any length of time. The pathological changes occur in all elements of the joint, and cartilage surface disruption is a constant factor. Recent investigations have proved that subchondral trabecular bone may be involved, and plays a significant role in the cartilage degeneration of OA (Burr and Schaffler, 1997). Epidemiological studies also show that subchondral bone sclerosis increases accompanying OA progression (Hannan et al., 1993). Subchondral bone sclerosis may not be required for initiation of cartilage fibrillation, but may be necessary for progression, and only changes in bone and calcified cartilage close to the joint are important for the disease process (Burr and Schaffler, 1997).

Some studies assume an abnormal low mineralization pattern, even though OA is associated with a thickening of the subchondral bone plate (Grynpas et al., 1991). Evidence accumulates to the fact that specific changes in the microstructure of subchondral trabecular bone in OA are consistent with an acceleration of bone turnover (Li and Aspden, 1997a), and an increasing severity of OA is correlated with joint narrowing (Dieppe et al., 1993). These structural changes occur through the action of osteoclasts and osteoblasts in selectively removing and adding bone. Structural changes in OA have also been described as differences in trabecular surface and shape compared with normal control groups (Fazzalari and Parkinson, 1997). Bone mineral density increases in both axial and peripheral skeleton with the OA progression (Dequeker, 1997).

One study has shown that, compared with normal control, apparent density is increased in OA, but decreased in OP, and tissue density is decreased in OA, but unchanged in OP (Li and Aspden, 1997a). Stiffness of OA subchondral bone increases more slowly as apparent density increases than does the stiffness for normal or OP groups. This suggests higher bone turnover rates and lower mineralization of bone tissue in OA (Li and Aspden, 1997a).

It is of interest that a recent biochemical study clearly has shown an abnormal trabecular bone collagen metabolism in OA (Mansell and Bailey, 1998). Bone collagen metabolism is increased in OA. The greatest changes in collagen metabolism and the hypomineralization of deposited collagen are found within the subchondral zone of osteoarthrotic femoral heads. Thus, bone collagen metabolism may be an important factor in the pathogenesis of OA, which deserves further attention.

Despite human studies have not revealed the hypothesis that the increasing bone metabolism primarily initiates cartilage destruction or vice versa. Recent studies on animal models, especially the macaque, have demonstrated that thickening of subchondral bone precedes fibrillation of the cartilage, which might be the results of increased bone resistance to compression (Carlson et al., 1996).

5.2.5 Properties of osteoporotic trabecular bone

The reduction in bone mass and the disruption in trabecular bone architecture are distinct characteristics of OP. The pathophysiology of primary OP is not clearly defined largely due to the heterogeneity of the disease. Most people consider OP as an age-related disease mainly occurring in women, but also in men (Seemán, 1997). Others suggest that primary OP is due to a greater biological aging rather than a specific disease process (Croucher et al., 1994). In adults, bone is continuously renewed through internal reorganization by the remodeling process: bone is turned over by localized osteoclastic bone resorption followed by osteoblastic bone formation (Steiniche, 1995).

The pathogenesis of bone loss is not completely understood. With aging and osteoporosis, a process of progressive reduced bone formation rather than increased bone resorption occurs, resulting in reduction of bone mass and structural changes. These changes may cause loss in mechanical strength, which are disproportionate to the reduction in bone mass alone. Since loss of bone mass does not entirely explain the loss of trabecular bone strength and the increase in fatigue fracture, other factors influencing bone quality and bone microstructure have to be considered.

Investigations show that significant changes occur in microstructure of OP patients compared with normal control, despite only a slight reduction in bone volume fraction occurs (Steiniche, 1995). These structural changes might be a consequence of trabecular plate perforations. The prevailing opinion is that bone loss in the vertebral centrum is accompanied by a reduction in trabecular number, by preferential resorption of horizontal trabeculae, and by hypertrophy of the remaining vertical trabeculae (Parfitt, 1984; Parfitt, 1987).

Fracture is the final clinical outcome of the osteoporotic process (Vaananen, 1991). OP would have little significance were it not for the associated fracture. The remaining bone tissue seems to be normal in composition, beside the reduction in bone mass (Nevitt, 1994). Osteoporotic fractures may occur as a result of minimal trauma or even spontaneously. Loss of bone mass contributing to bone fragility, deterioration in bone structure, and quality changes in bone matrix all play a significant role in the pathogenesis of osteoporotic fractures (Parfitt et al., 1983; Parfitt, 1987; Vaananen, 1991).

5.2.6 Changes in the properties of medial and lateral condyles

Hvid (1988) has clearly demonstrated that medial tibial condyle is a large high strength area with maximal strength centrally and slightly anteriorly, and lateral condyle being a restricted area of relatively high strength posteriorly. The strength of the medial condyle is significantly larger than that of lateral condyle. It has also been shown that strength at both condyles is reduced toward the periphery area, and at the margins of the condyles and intercondylar region being the lowest. This pattern of axial compressive strength and stiffness distribution shows the same at the normal human tibia (Hvid, 1988). Furthermore, the average values of densities (apparent density, apparent ash density, collagen density and volume fraction) from the medial condyle were significantly larger (14–26%) than those from the lateral condyle (Ding et al., 1997). These results are in consistence with higher loading distribution at the medial condyle.

OA, whatever caused, always initiates on the medial condyle of tibia. In the early-stage OA, changes predominantly occur on central medial condyle resulting in disruption of articular cartilage and sclerosis of underlying bone, while lateral condyle is virtually unaffected. This phenomenon may be explained by strength distribution pattern in tibial condyles, as is both seen in human tibia OA (Ding et al., 1998b; Matsui et al., 1997) and animal OA model (Carlson et al., 1996) Only in moderate or severe stage, can changes actually occur in lateral condyle.

The strength distribution pattern may change in severe OA (Hvid and Hansen, 1986), in which the mal-alignment of loading force occurs mainly due to the subchondral bony changes. Using histological and histomorphometric methods, Matsui et al. (Matsui et al., 1997) found a parallel relationship between the bone volume/bone formation rate and the cartilage degeneration in both medial and lateral condyles of knee OA. They assumed that the remodeling of underlying subchondral bone influenced the degeneration of joint cartilage (Matsui et al., 1997). Trabecular orientation in OA was found to be more vertical or perpendicular to the articular surface than normal control, especially in the medial condyle where trabeculae are close to the articular cartilage surface (Kamibayashi et al., 1995a).

It has been demonstrated that aging resulted in different pattern of microstructural variations in human tibial cancellous bone (Ding et al., 2000). In normal individual, density and strength differed between condyles, whereas ultimate strain and failure energy showed no significant difference between both condyles (Ding et al., 1997). Three-dimensional structural parameters, such as connectivity and volume fraction of the medial condyle differed significantly from those of the lateral condyle, whereas anisotropy and bone surface density did not show significant difference (Ding et al., 2000). Despite significant differences in some properties between the condyles, age-related trends in the microstructural properties were the same for both condyles. Therefore, the average values of microstructural properties from the medial and the lateral condyles represented the age-related trends for both condyles (Ding et al., 2000). The pattern of 3-D structural change in OA and OP remains to be clarified.

5.3 Age variations in the properties of the cartilage-bone complex

5.3.1 Age variations in mechanical properties of the normal tibial cartilage-bone complex

Articular cartilage is a structure rather than a material (Jeffery et al., 1991), which makes it impossible to remove small samples for testing without disrupting its structural integrity (Obeid et al., 1994). It has been suggested (Obeid et al., 1994) that a cartilage plug of 8 mm in diameter offered a specimen with a rather reasonable flat surface and minimal disrupted collagen network. Cylindrical cartilage-bone complex specimens (diameter 7.5 mm, without sectioning subchondral bone plate) seem satisfactory to interpret the local properties without too much disruption of its structure (Ding et al., 1998a; Ding et al., 1998b). There are also many factors, such as storage and temperature, other than testing itself, that might have some effects on the mechanical properties of cartilage. One must be careful to compare results obtained from different testing conditions.

Young's modulus of bone from combined testing technique (Ding et al., 1998a) seems higher than those reported in studies on isolated trabecular bone from the human proximal tibia (Linde, 1994). This is probably due to the constraining effect of the subchondral bone plate. Linde and Hvid (Linde and Hvid, 1989) found that fixation of trabecular bone specimens to a thin layer of bone cement increased the modulus by 40%. Therefore, it is likely that the subchondral bone plate has a similar effect and thus increases the modulus of both cartilage and trabecular bone.

It seems that a minor overestimation occurs due to the inclusion of the thin, but stiff layer (about 1 mm in thickness) of the subchondral bone plate (Røhl et al., 1997). The magnitude of this overestimation has been estimated by Røhl et al. (1997) to be approximately 15% as regards the cartilage part and 5% as regards the bone part. The reproducibility for testing of cartilage-bone complex is excellent, therefore the results are comparable (Røhl et al., 1997).

The mechanical properties of articular cartilage did not depend significantly on cartilage thickness (Akizuki et al., 1986; Athanasiou et al., 1991; Ding et al., 1998a). By testing cartilage alone, investigators have reported a significant age-related decline in the tensile modulus of human talus cartilage (Kempson, 1991), in the equilibrium modulus of human patella cartilage (Armstrong and Mow, 1982), and in the tensile modulus of human femoral head cartilage (Kempson, 1991). Swann and Seedhom (Swann and Seedhom, 1993), on the other hand, reported no age dependence on the compressive modulus of femoral and tibial cartilage or talar and tibial cartilage.

It has been shown that from 7–90 years, the tensile stress of superficial zone decreased from 33 to 10 MPa, and the tensile stiffness from 150 to 80 MPa. From 7 years, the tensile stress of mid-zone decreased from 32 to 2 MPa at 85 years and the tensile stiffness from 60 to 10 MPa at 60 years (Kleerekoper et al., 1985).

Compared with tibial trabecular bone (Ding et al., 1997), the Young's modulus of cartilage also shows an initial increase until 29 years, and is constant between 30 and 50, and declines thereafter (Ding et al., 1998a). Kempson (Kempson, 1982) reported that the tensile stiffness of the superficial layer of cartilage in the femoral condyle increased with age to a maximal value in the third decade and decreased thereafter, while the stiffness of the deep zone decreased continuously with age. Swann and Seedhom (1993) reported no significant age dependence of the compressive stiffness of knee cartilage; however, only 13 knee joints with age ranging from 14–59 years were used in their study. In fact, a latter study shows that the Young's modulus of cartilage (despite the initial increase) is relatively constant between the second up to the fifth decade. The decline in the Young's modulus of cartilage happens first after 50 years (Ding et al., 1998a).

It is an important finding for articular cartilage that the relative energy loss showed no substantial difference from the second decade up to the eighth decade. Therefore, this consistent character of the relative energy loss may indicate that cartilage preserves its energy absorption capacity throughout life.

Young's modulus of bone from combined testing is 11 times higher than that of cartilage ($p<0.001$) (Ding et al., 1998a). This result is confirmed by the previous findings of Radin and Paul (1970) and Radin et al. (1970). They reported articular cartilage per unit of volume reduced the peak load 10 times more than subchondral bone. Nevertheless, due to the large amount of bone, cartilage contributed only in a minor way to reduce the peak force.

It is of interest to note that cartilage and bone show a similar pattern in the variations of mechanical properties with age. It is also of interest that the Young's moduli are significantly correlated between cartilage and bone. These findings might suggest that articular cartilage and subchondral trabecular bone function as a unit to mechanical loading.

5.3.2 Changes in the mechanical properties of the osteoarthrotic cartilage-bone complex

The OA specimens used for in vitro study were often classified by the degeneration changes at autopsy based on morphology. These changes were often histologically graded according to Mankin's criteria (Mankin et al., 1971). Brocklehurst et al. (Brocklehurst et al., 1984) and van Valburg et al. (van Valburg et al., 1997) found a good correlation between the results of histology and the visual appearance of human knee articular cartilage observed at autopsy. These studies concluded that the changes in cartilage surface were suitable for studying the process of cartilage degeneration in OA. The lateral condyle of the same tibia is often used as a comparison group. However, the lateral condyle may also be affected by degeneration process, even though it behaves the same mechanical pattern as those of the normal age-matched groups (Ding et al., 1998b). Thus, the lateral condyle used as comparison group may not be a good control group.

The stiffnesses of normal cartilage and bone from medial tibial condyles are generally larger than those from lateral condyle (Ding et al., 1997; Ding et al., 1998a; Hvid and Hansen, 1985). However, during the development of early-stage OA, both OA cartilage and bone showed a reduction in stiffness compared to the lateral comparison and medial age-matched groups. These results support previous findings based on testing of cartilage (Obeid et al., 1994) or bone (Hvid and Hansen, 1986) separately. It has been found that both cartilage and subchondral bone are mechanically inferior to normal in early-stage OA (Ding et al., 1998b), and OA trabecular bone differs significantly from normal (Li and Aspden, 1997a).

Normal mechanical loading plays an important role in the maintenance of normal articular cartilage. It has been demonstrated that stiffnesses correlate significantly between normal cartilage and bone (Ding et al., 1998a). Hence, it is an interesting finding that the stiffness correlation between cartilage and bone for the OA group was lost, whereas for the other three groups, these correlations between cartilage and bone remained significant (Ding et al., 1998b). Therefore, it is reasonable to suggest that, due to early-stage OA, the cartilage and subchondral bone have lost their unit function in response to mechanical loading (Ding et al., 1998b).

5.3.3 Changes in the properties of osteoarthrotic cartilage

The initiation and progression of cartilage damage are distinct phenomena of OA, which have been proposed to be multifactorial, such as biomechanical (Radin et al., 1970; Radin and Rose, 1986; Radin et al., 1987), biochemical (Brocklehurst et al., 1984; Mansell and Bailey, 1998), and pathological changes (Mankin et al., 1971). A strong correlation between advancing aging and the prevalence of OA, and important age-related changes in the function of chondrocytes may suggest age-related changes in the cartilage that may contribute to the development and progression of OA (Buckwalter and Mankin, 1998).

It has been found that the water content of human OA articular cartilage was above normal and was highly correlated with the intrinsic equilibrium modulus and permeability (Armstrong and Mow, 1982). As the water content increases, the matrix of cartilage tissue becomes more permeable and softer, and the decrease in modulus is balanced by the increase in permeability. In a canine OA model, breakdown of the collagen network, decrease in tensile stiffness and increase in swelling were observed (Altman et al., 1984). It has also been suggested that the microstructural alterations of collagen-proteoglycan solid matrix in canine OA may play a more important role than the composition in determination of its mechanical properties (Guilak et al., 1994; Setton et al., 1993).

The structural integrity of the solid matrix, such as collagen and proteoglycans, provides cartilage the ability to withstand mechanical loading. The slight fissuring of the cartilage superficial zone due to early-stage OA is the result of damage to its collagen network, and hence, the cause of the reduction of cartilage stiffness. The marked thinning of cartilage is one of the early features of OA. It is, therefore, not surprising that OA cartilage shows reduction in the thickness. However, the cartilage thickness did not correlate with the cartilage stiffness (Athanasiou et al., 1991; Ding et al., 1998). It appears that the changes in cartilage due to early OA mainly cause disruption of cartilage collagen fiber network (Guilak et al., 1994), and consequently disrupt the unit function of cartilage and subchondral bone in response to mechanical loading (Ding et al., 1998b).

Conclusion

6.1 Variations in the properties of trabecular bone (studies I & II)

The mechanical properties, collagen concentration, apparent density, apparent ash density and collagen density of human tibial trabecular bone have significant relationships with age. Trabecular bone is tougher in the younger age. Tissue density and mineral concentration are constant throughout life. Collagen density was found to be the single best predictor of failure energy, and collagen concentration was found to be the only predictor of ultimate strain. The decrease in mechanical properties of trabecular bone such as Young's modulus and ultimate stress mainly is a consequence of the loss of trabecular bone substance (quantity), rather than a decrease in the quality of substance itself (Ding et al., 1997).

All structural parameters of human tibial trabecular bone have significant correlations with age, except connectivity. Age-related changes in the microstructural properties had the same trends for both medial and lateral condyles of the tibia. The observed increase of anisotropy may be interpreted as the consequence of structural adaptation secondary to age-induced bone loss. The aging trabeculae align more strongly to the primary direction, which is parallel to the longitudinal loading axis of the tibia (Ding et al., 2000).

6.2 Variations in the properties of cartilage-bone complex (studies III & IV)

The mechanical properties of the normal cartilage and bone vary with age and function simultaneously to mechanical loading (Ding et al., 1998a).

Both cartilage and bone in early-stage OA are mechanically inferior to normal, and OA cartilage and bone have lost their unit function to mechanical loading (Ding et al., 1998b).

Summary

Initiated and motivated by clinical and scientific problems such as age-related bone fracture, prosthetic loosening, bone remodeling, and degenerative bone diseases, much significant research on the properties of trabecular bone has been carried out over the last two decades. This work has mainly focused on the central vertebral trabecular bone, while little is known about age-related changes in the properties of human peripheral (tibial) trabecular bone. Knowledge of the properties of peripheral (tibial) trabecular bone is of major importance for the understanding of degenerative diseases such as osteoarthrosis and osteoporosis, and for the design, fixation and durability of total joint prosthesis.

The specific aims of the present studies were: 1) to investigate normal age-related variations in the mechanical, physical/compositional, and structural properties of human tibial trabecular bone; and 2) to investigate the age-related and osteoarthrosis-related changes in the mechanical properties of the human tibial cartilage-bone complex; and 3) to evaluate mutual associations among various properties.

Normal specimens from human autopsy proximal tibiae were used for investigation of age variations in the properties of trabecular bone and the cartilage-bone complex, and osteoarthrotic specimens were used for the investigation of changes in the mechanical properties of the cartilage-bone complex induced by this disease process. The mechanical properties and physical/compositional properties of trabecular bone were quantified by means of standard techniques, and trabecular bone structure was quantified by means of unbiased three-dimensional methods.

The present study demonstrated that the mechanical properties, such as Young's modulus, ultimate stress, ultimate strain and failure energy, and the densities, such as apparent, apparent ash and collagen densities of human tibial trabecular bone have significant relationships with age. Tissue density and mineral concentration remain constant throughout life. Trabecular bone is tougher in the younger age, i.e. fracture requires more energy. Collagen density was the single best predictor of failure energy, and collagen concentration was the only predictor of ultimate strain. The decrease in mechanical properties of trabecular bone mainly is a consequence of the loss of trabecular bone substance.

This study showed that the degree of anisotropy (preferential orientation of trabeculae), mean marrow space volume, and bone surface-to-volume ratio increased significantly with age. Bone volume fraction, mean trabecular volume, and bone surface density decreased significantly with age. Connectivity did not have a general relationship with age, yet a trend exists. Age-related changes in the microstructural properties had the same trends for both medial and lateral condyles of the tibia. The observed increase of anisotropy may be interpreted as the consequence of structural adaptation secondary to age-induced bone loss. The aging trabeculae align more strongly to the primary direction, which is parallel to the longitudinal loading axis of the tibia.

The mechanical properties of the normal cartilage and bone vary with age and respond simultaneously to mechanical loading. Both cartilage and bone in early-stage OA are mechanically inferior to normal, and OA cartilage and bone have lost their unit function to mechanical loading.

Future research

Interesting areas remain for further study:

The present study has provided the basic information and a better understanding of the age-related variations in the mechanical, physical/compositional and structural properties of trabecular bone for both basic research and clinic. It seems very important to further investigate the changes in the properties under pathological conditions such as osteoarthrosis and osteoporosis. Actually, a research protocol has been initiated, and the project is progressing well. The perspectives of these continuing approaches, based on the recent investigations, are to seek further insight into a better understanding of age-related variations in the properties of trabecular bone and a better understanding of some very frequent degenerative diseases, such as osteoarthrosis.

The specific purposes of the further study are:

1) to assess mutual associations between various structural and compositional properties of normal human peripheral trabecular bone and their influence on mechanical properties; and

to quantify degree of mineralization in normal and OA trabecular bone, and its influence on mechanical properties.

2) to quantify the changes in 3-D microstructure of peripheral trabecular bone under pathological conditions; and changes in the associations among mechanical, physical/compositional, and structural properties in pathological trabecular bone.

3) to investigate the changes in trabecular microstructure in various stages of OA in guinea pigs, one of the best models for investigating spontaneous OA.

4) to investigate age-related changes in the microstructral properties of human central vertebral trabecular bone, especially changing in the properties of skeletal immature bone tissue.

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