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Changes in spatial collagen content and collagen network architecture in porcine articular cartilage during growth and maturation

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Summary

Objectives: The present study was designed to reveal changes in the collagen network architecture and collagen content in cartilage during growth and maturation of pigs.

Methods: Femoral groove articular cartilage specimens were collected from 4-, 11- and 21-month-old domestic pigs ($n = 12$ in each group). The animal care conditions were kept constant throughout the study. Polarized light microscopy was used to determine the collagen fibril network birefringence, fibril orientation and parallelism. Infrared spectroscopy was used to monitor changes in the spatial collagen content in cartilage tissue.

Results: During growth, gradual alterations were recorded in the collagen network properties. At 4 months of age, a major part of the collagen fibrils was oriented parallel to the cartilage surface throughout the tissue. However, the fibril orientation changed considerably as skeletal maturation progressed. At 21 months of age, the fibrils of the deep zone cartilage ran predominantly at right angles to the cartilage surface. The collagen content increased and its depthwise distribution changed during growth and maturation. A significant increase of the collagen network birefringence was observed in the deep tissue at the age of 21 months.

Conclusions: The present study revealed dynamic changes of the collagen network during growth and maturation of the pigs. The structure of the collagen network of young pigs gradually approached a network with the classical Benninghoff architecture. The probable explanation for the alterations is growth of the bone epiphysis with simultaneous adaptation of the cartilage to increased joint loading. The maturation of articular cartilage advances gradually with age and offers, in principle, the possibility to influence the quality of the tissue, especially by habitual joint loading. These observations in porcine cartilage may be of significance with respect to the maturation of human articular cartilage.

Key words: Articular cartilage, Polarized light microscopy, Fourier transform infrared imaging, Collagen network, Growth and maturation.

Introduction

The unique biomechanical properties of articular cartilage result from the complex architecture of the collagen network and the non-homogeneous spatial distribution of collagen, proteoglycans (PGs) and water in the tissue. It is well documented that the average biochemical composition of cartilage changes gradually with age[1–3] and concomitantly, there are also changes in the biomechanical properties of cartilage[4–6]. Previous studies have mostly been concerned with compositional and the corresponding biomechanical changes without taking into account the architectural changes occurring in the collagen network. Recent modeling studies have supplemented our knowledge on the significance of the collagen network in determining the biomechanical properties of articular cartilage[7,8]. Biomechanical models have revealed that alterations of the collagen network directly influence the biomechanical behavior of the cartilage. It is thus evident that the biomechanical behavior of articular cartilage cannot be fully characterized by its composition without taking into account the role of the architecture of the collagen network.

After birth, articular cartilage is rich in PGs and when viewed under the microscope, the tissue appears homogeneous. At this stage, the collagen network is not organized in a zonal manner. Further, different joint areas have a similar type of cartilage tissue. This means that local adaptation to intrinsic or external determinants such as joint loading has not yet taken place[9]. Recent histomorphometric and magnetic resonance imaging studies have pointed to significant alterations of the collagen network taking place during skeletal maturation[10,11]. It has been proposed that the adult articular cartilage structure is gradually formed by the division and activity of the stem (progenitor) cells of the cartilage surface and that this involves simultaneous tissue resorption and structural neoformation[10]. In magnetic

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resonance images, the appearance of the T_2-weighted images, known to relate closely to collagen tissue architecture, changes with age. The multilaminar appearance which is common at a young age becomes transformed to a trilaminar, adult phenotype as skeletal maturity is achieved[11]. This strongly suggests that the original collagen network of cartilage is gradually replaced by a new collagen framework during skeletal maturation[10,11].

The composition and the biomechanical properties of articular cartilage vary in different joints and in different joint areas[12,13]. Also changes in joint loading conditions, e.g., due to strenuous exercise or immobilization, influence both the biochemical composition and the biomechanical properties of the articular cartilage[14,15]. These changes are indicative of the presence and impact of varying degrees and types of physical stress in different joints. In addition, there are zonal and maturational differences in the superficial and deep zone chondrocytes[16–19]. Recently, the superficial zone of calf articular cartilage was shown to contain a population of (progenitor) cells[20].

These developmental changes support the idea that cartilage undergoes major changes during maturation. This opens the possibility that physical exercise during growth and adolescence can improve the properties of cartilage and even prevent the appearance of osteoarthrosis in older age[21]. It is not clear at what age the cartilage is most responsive to external joint loads and weightbearing. Enzymes participating in the modification of articular cartilage exhibit high activities in fetal and adolescent cartilage but their activity diminishes as growth and maturation proceeds[22]. Thus the most dynamic alterations of articular cartilage appear to occur during skeletal growth. Once the skeletal maturation has been attained, the cartilage tissue maintains a stable highly unique structure with minor later modifications[23–25].

The present study was designed to characterize the development and changes in the architecture of the collagen network of porcine articular cartilage during growth and maturation. Polarized light microscopy and Fourier transform infrared imaging spectroscopy (FT-IRIS) enabled qualitative and quantitative analysis of the spatial alterations of the collagen network.

Methods

ANIMALS AND THE PREPARATION OF THE SAMPLES

Domestic female pigs were used as experimental animals. The pigs were housed in the National Laboratory Animal Center, University of Kuopio, in individual pens with a floor area of $2 \times 2\ m^2$. The animals, kept on standard diet, were euthanized at the age of 4, 11 and 21 months when the weights of the animals were approximately 80, 150, and 200 kg, respectively. The Animal Care and Use Committee of the University of Kuopio approved the experimental design of this study. The cartilage samples were prepared by drilling cartilage–bone plugs from knee joints (lateral facet of the femoral trochlea) ($n = 12$ per group) (Fig. 1). The harvested specimens were fixed in phosphate buffered 10% formalin, pH 7.4. Decalcification was carried out with 10% of ethylenediaminetetraacetic acid (EDTA) in phosphate buffer, pH 7.4, containing 10% formalin for 14 days. The specimens were dehydrated in an ascending series of alcohol solutions and treated with xylene prior to embedding in paraffin as described earlier[26]. Five-μm-thick histological sections were prepared for polarized light microscopy and FT-IRIS. Sections were deparaffinized and treated with hyaluronidase, 1000 U/ml at 37°C for 18 h (bovine testicular hyaluronidase, from Sigma) to remove PGs from the tissue sections[26]. Enzymatic removal of PGs was controlled with staining of glycosaminoglycans with Safranin-O stain. Only faint color was left after hyaluronidase treatment in contrast to the intensive deep red staining of untreated sections. This indicated that the majority of the PGs was removed during the procedure[26]. The distribution profiles of collagen in cartilage sections were determined from hyaluronidase-treated sections. However, untreated sections were used for the determination of the collagen and PG contents around the chondrocyte lacunae.

POLARIZED LIGHT MICROSCOPY

Polarized light microscopy was used for the determination of the collagen network birefringence, collagen fibril parallelism and collagen fibril orientation[27–29]. The measurement system was based on a scientific grade polarized light microscope, Leitz Ortholux II POL (Leitz Wetzlar, Wetzlar, Germany). The optical components represented precision quality optics. The microscope was modified for computation of the Stokes parameters[27,28]. The polarizers were placed in computer-controlled rotation tables. Two separate monochromators (591.4 ± 10 nm, Schott, Germany and 594 ± 3 nm, XLK10, Omega Optical Inc., Brattleboro, VT, USA) were used to adjust the final measuring wavelength ($\lambda = 594 \pm 3$ nm). A Peltier-cooled, high-performance CCD camera (Photometrics SesSys, RoperScientific, Tucson, AZ, USA) was used for the signal detection. Polarizers and image acquisition systems were controlled with a Macintosh G4 computer (Apple Computer, Cupertino, CA, USA) utilizing the scripting capabilities of IpLab 3.5.5 software (Scanalytics Inc., Fairfax, VA, USA)[29].

In polarized light microscopy, seven images were captured and used for the calculation of the orientation independent parameters. Starting from the alignment position (=0°), 15° stepwise rotations were made of the polarizer pair. Thus, images were recorded at 0, 15, 30, 45, 60, 75 and 90° polarizer pair positions, and an additional image was taken at the 90° position after placing the $\lambda/4$ phase shift plate into the light path. Pixel by pixel background-corrected 0, 45, 90, and the 90° images with a $\lambda/4$ phase shift plate were used for the calculation of the Stokes parameters. For determination of the birefringence, parallelism (anisotropy) and orientation parameters, also 15, 30, and 75° images were used (Fig. 2).

The orientation independent birefringence was calculated for each sample. Maximum theoretical birefringence was derived from the background-corrected images at 0, 15, 30, 45, 60, 75 and 90°. The maximal signal was determined using the least square fitting procedure for each image pixel. The fitting procedure enables determination of the birefringence regardless of the collagen fibril direction. Birefringence is a parameter that indicates the combined effect of collagen content and collagen network organization[29].

The parallelism index was calculated from the detected maximum and minimum signals[29]. The parallelism index indicates the extent to which the collagen fibrils are running in parallel in each image pixel, i.e., whether fibrils are running in the same direction or whether they take different paths in the tissue.

The orientation of collagen fibrils was calculated from the Stokes parameters by the determination of the angle of the polarization ellipse[29]. A defined orientation represents the average orientation of the fibrils within single pixels of a region and, therefore, the orientation of individual fibrils is not revealed.

The polarized light microscopic images were rescaled to a lower resolution and 5×5 median filtering was done before the final calculations. Each cluster that underwent filtering corresponded to approximately 50×50 μm. Cartilage lacunae cause bias during calculations and, therefore, those areas were excluded from the final data. Filtering was conducted to avoid the effects of different cell densities on the final calculations. In particular, the cell lacunae affect the calculation of both the fibril orientation and the parallelism index.

FT-IRIS

The collagen content of articular cartilage was estimated with FT-IRIS measurements[30,31]. Five-μm-thick tissue sections were imaged with Perkin Elmer Spotlight 300 instrument (Perkin Elmer, Waltham, MA, USA). The sections were placed on 2-mm-thick barium fluoride-windows. The hyaluronidase treatment ensured that the spectral information was as specific as possible for collagen. FT-IRIS data were collected using 8 cm^{-1} spectral resolution and 6.25 μm pixel resolution. Two repeated scans were performed and averaged for each specimen. The spectral information from a region between 3600 and 700 cm^{-1} was collected. The collagen content was estimated by integration of the amide I region (1710–1610 cm^{-1})[30].

Additional tests were conducted to estimate possible effects of the collagen fibril orientation on the analysis of collagen content. Partially polarized IR-light has been suggested to generate artifacts to the amide I and amide II peak measurements when highly anisotropic fibrillar material, such as cartilage, is measured[32]. To test this hypothesis, a cartilage specimen was measured three times while the sample was rotated 45° after each measurement. Amide I peak maps and distribution profiles were calculated from surface to deep cartilage for each measurement. No significant alterations were observed between the measurements indicating that differences of the collagen fibril orientation between the studied groups were not affecting the FT-IRIS measurements.

STATISTICAL ANALYSIS

Statistical significance of the differences between experimental groups was tested using the non-parametric Kruskall–Wallis test (SPSS 11.5 software, SPSS Inc., Chicago, IL, USA). Contrast analysis between the age points was undertaken with a *post hoc* test for multiple comparisons. The limit of statistical significance was set to $P = 0.05$.

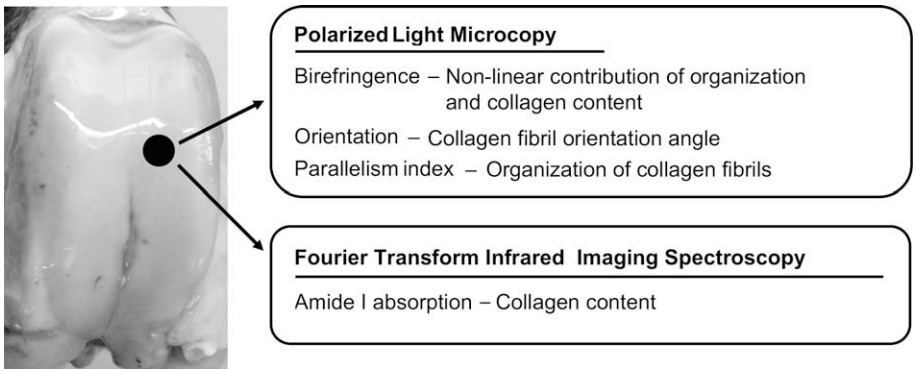

Fig. 1. A characteristic image of the porcine femoral groove with the sample site on the lateral facet. A cartilage–bone cylinder was taken and processed for polarized light microscopy and FT-IRIS.

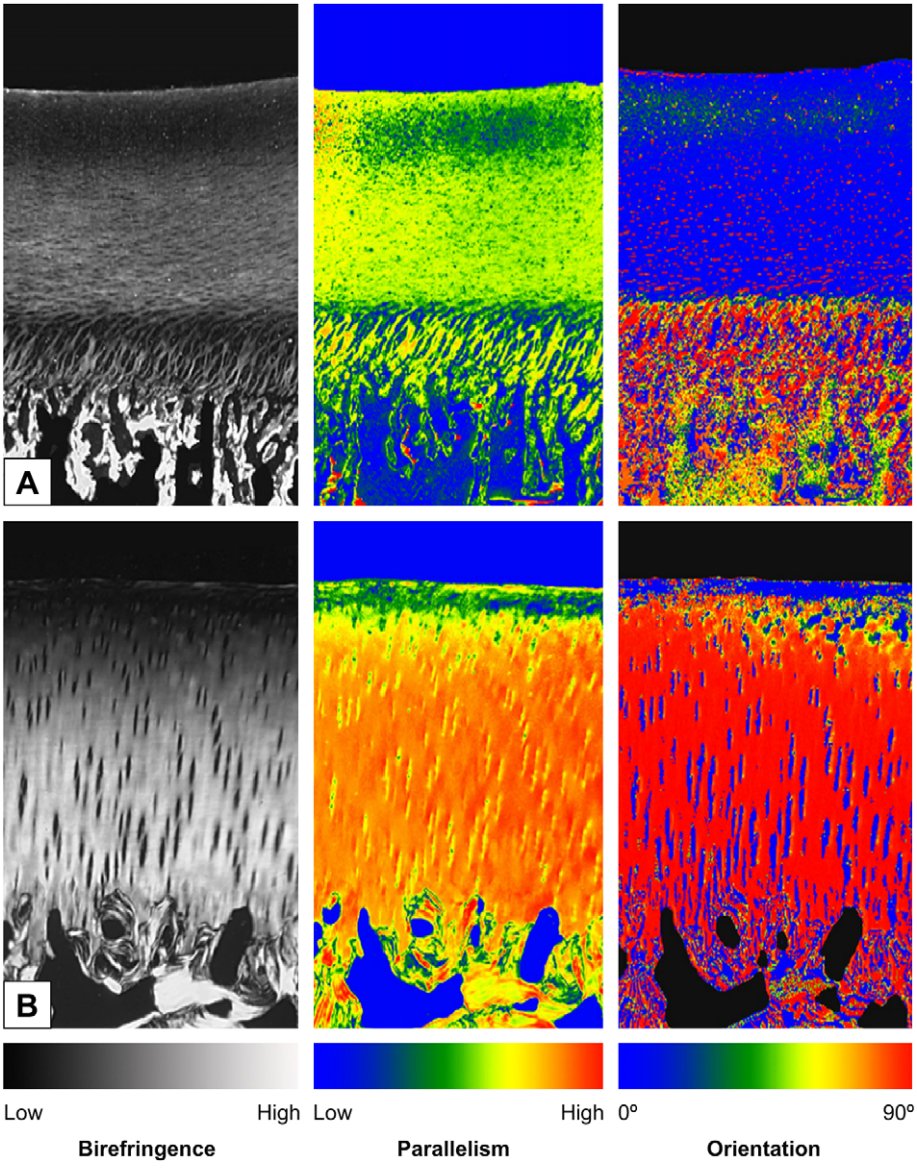

Fig. 2. Polarized light microscopy images demonstrating the alterations in the collagen fibril network in young (4-month-old) (A) and more mature (21-month-old) (B) porcine cartilage. Collagen birefringence, local fibril parallelism and fibril orientation in relation to the cartilage surface, determined by quantitative polarized light microscopy, change gradually with growth and tissue maturation.

Results

The articular cartilage collagen network, as assessed by polarized light microscopy and FT-IRIS parameters, underwent significant changes during growth and maturation from the age of 4–21 months (Fig. 2). The cartilage thickness gradually decreased as the animals matured ($P < 0.01$). The average thickness was 2294 ± 569, 1141 ± 238 and 790 ± 117 μm for 4-, 11- and 21-month-old animals, respectively (Fig. 3).

Microscopical assessment was first performed by analyzing the cartilage specimens of each group in 10% fractions from the superficial to the deep cartilage. The collagen content increased significantly during cartilage growth and maturation [Fig. 4(A)]. The average collagen content (amide I absorption) increased gradually as maturation proceeded. The collagen content underwent a major increase between the 4- and 11-month age points ($P < 0.01$). The cartilage of the youngest animals revealed high collagen content in the upper part of the deep zone, whereas in the older animals, the cartilage showed a more substantial increase in the collagen content with tissue depth [Fig. 4(A)]. In general, the collagen content increased gradually throughout the cartilage thickness. The greatest increase was observed in the deep cartilage (Fig. 4A). The average amide I absorption was 0.18 ± 0.04, 0.27 ± 0.04 and 0.33 ± 0.06 in the 4-, 11- and 21-month-old animals, respectively [Fig. 5(A)].

At the age of 4 months, the collagen fibrils had a predominantly parallel orientation with the cartilage surface throughout the cartilage [Fig. 2, Fig. 4(B)]. The organization of collagen fibrils changed as the animals reached the age of 11 months. At this timepoint, a decrease in the parallelism index took place [Figs. 4(C) and 5(C)] and the collagen network apparently exhibited a transition phase from a less organized architecture toward the adult phenotype (Fig. 4). At the age of 21 months, the collagen network exhibited the classical Benninghoff-type arrangement[33] [Fig. 2(B)]. At this stage, the superficial zone collagen fibrils ran parallel to the cartilage surface. Then, the fibrils turned into the deep cartilage to run at right angles to the surface [Figs. 2(B) and 4(B)]. The average fibril orientation in cartilage

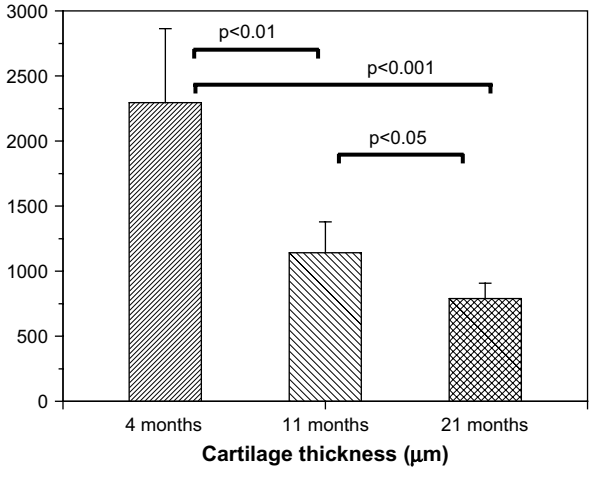

Fig. 3. Cartilage thickness is presented as group averages (mean ± S.D.). The thickness shows a gradual decrease with age. Statistical differences were determined by the non-parametric Kruskall–Wallis test. The contrasts between the different age points were tested with a *post hoc* test. Statistically significant differences are indicated.

was at 27.3 ± 8.6, 47.8 ± 21.4 and $75.9 \pm 5.5^\circ$ for 4-, 11- and 21-month-old animals, respectively (0° represents the parallel with the cartilage surface) [Fig. 5(B)]. The parallelism index indicated a highly organized collagen network both in 4- and 21-month-old animals. At the age of 11 months, however, there was a significant decrease in the degree of fibril organization ($P < 0.001$) [Figs. 4(C) and 5(C)]. The average parallelism indices were $72.4.3 \pm 13.6$, 53.7 ± 5.3 and 70.8 ± 3.4 in 4-, 11- and 21-month-old animals, respectively [Fig. 4(C)]. The birefringence increased between the timepoints of 11 and 21 months ($P < 0.001$), but no significant differences were found when 4- and 11-month-old animals were compared [Fig. 5(D)]. The birefringence increased especially in the deep zone cartilage [Fig. 4(D)]. The distribution profile of the birefringence throughout the cartilage thickness was very different from that of both parallelism and orientation. Birefringence could not distinguish the 4- and 11-month-old tissues from each other, even though there was a clear decrease in the degree of parallelism between these timepoints [Figs. 4 (C,D) and 5(C,D)].

The cartilage showed thinning during growth and maturation. To reveal and examine in more detail the changes in cartilage tissue, the collagen fibril network properties were also studied by analyzing separately the most superficial 800 μm tissue layer at each timepoint. At 21 months of age, the average cartilage thickness of the cartilage was 800 μm. Significant changes were again observed in all measured parameters. A gradual increase in the collagen content was observed with maturation of the tissue throughout the tissue when 4-month-old animals were compared with their 11- and 21-month-old counterparts ($P < 0.01$) [Fig. 6(A)]. The average amide I absorption values were 0.18 ± 0.04, 0.26 ± 0.04 and 0.32 ± 0.07 in the 4-, 11- and 21-month-old animals, respectively. Highly organized collagen fibrils, oriented tangentially to the surface, were replaced with a collagen network that was structured zonalwise, i.e., followed closely the classical Benninghoff model (Figs. 2 and 6)[33]. Changes in collagen content, parallelism and orientation were observed in all three cartilage zones (superficial, intermediate and deep cartilage) whereas birefringence only changed in the deep zone of 21-month-old pigs (Fig. 6). The average values for collagen fibril orientation were 26.3 ± 14.0, 43.0 ± 20.9 and $75.8 \pm 5.1^\circ$ for 4-, 11- and 21-month-old animals, respectively. Changes of the collagen fibril orientation were significant between 4- and 21-month-old animals ($P < 0.001$) and 11- and 21-month-old animals ($P < 0.05$). The parallelism index displayed a significant alteration only between the 11- and 21-month-old animals ($P < 0.01$). The average parallelism index values were 66.0 ± 21.0, 51.8 ± 5.2 and 70.7 ± 30.8 for 4-, 11- and 21-month-old animals, respectively. The birefringence increased significantly for 21-month-old animals ($P < 0.01$), but no statistically significant difference was observed between 4- and 11-month-old animals. The average birefringence values were 0.45 ± 0.12, 0.50 ± 0.13 and 1.48 ± 0.55 ($\times 10^{-3}$) for 4-, 11- and 21-month-old animals, respectively.

FT-IRIS results showed that the area adjacent to cell lacunae, i.e., the territorial matrix, contained less collagen and more PGs than the interterritorial matrix (Fig. 7)[34,35]. Characteristically, 4-month-old pigs exhibited a higher cell density than the 21-month-old animals [Fig. 7(A,B)]. During maturation, the depth-dependent collagen content as well as that around the cell lacunae altered characteristically, indicating growth-related alterations also in the microenvironment around the cell lacunae.

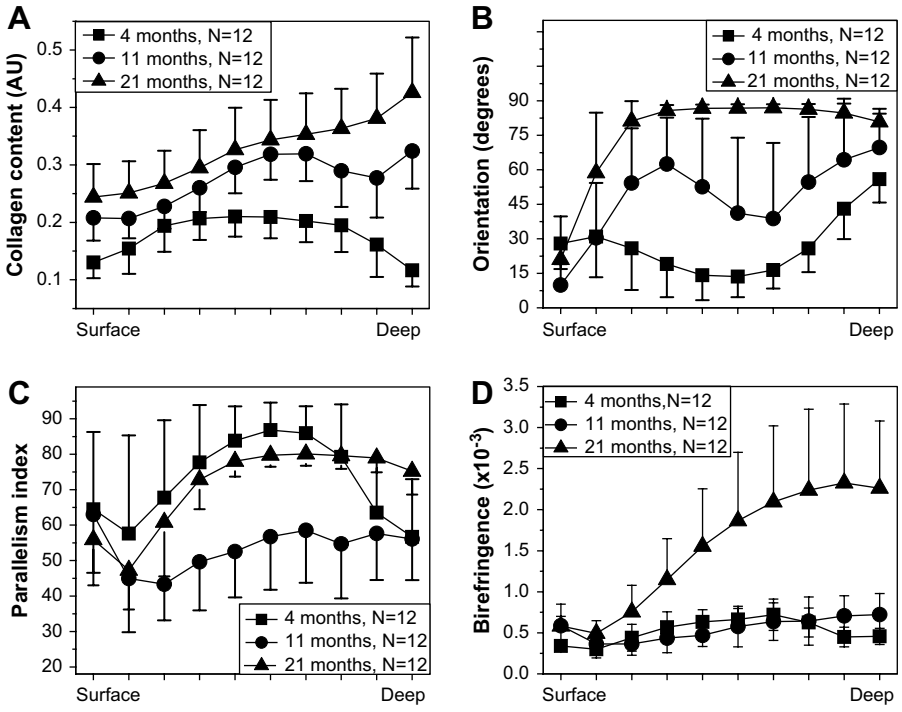

Fig. 4. Development of collagen content (amide I absorption, AU = absorbance unit) (A), collagen fibril orientation (B), parallelism index of collagen (C), and collagen birefringence (D) in articular cartilage of growing pigs. Depthwise profiles from surface to deep zone are normalized according to thickness, i.e., each fraction on the *x*-axis represents 10% of the cartilage thickness. Mean ± S.D.

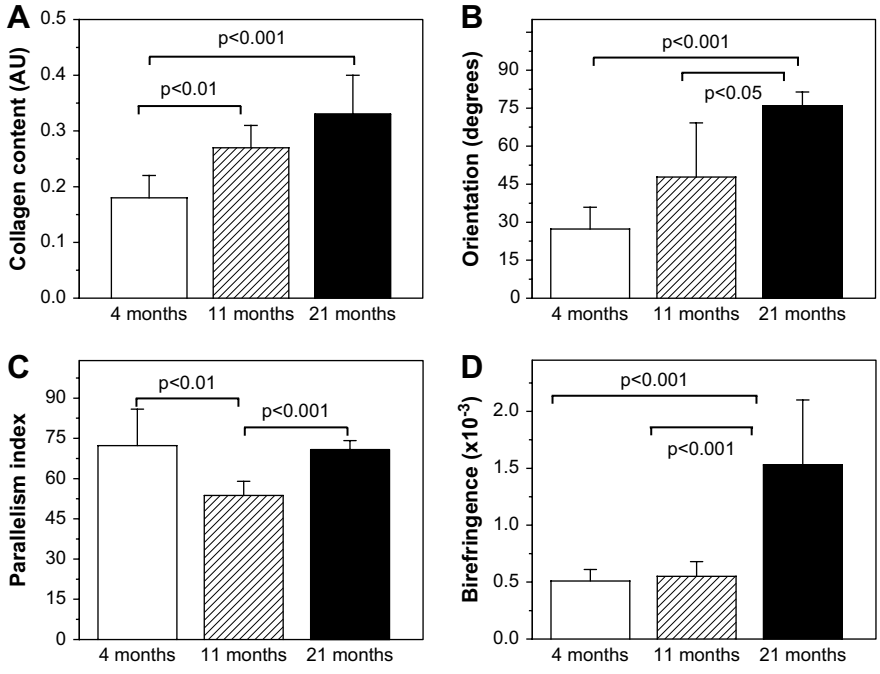

Fig. 5. Collagen content (amide I absorption, AU = absorbance unit) (A), collagen fibril orientation with respect to cartilage surface (B), parallelism index of collagen (C), and collagen birefringence (D) are presented. Each parameter was calculated across the cartilage depth (from surface to cartilage–bone junction) using a 500 μm wide region of interest. An average value was calculated for each row of profiles from the superficial cartilage to the cartilage–bone junction (Mean ± S.D). Statistical differences were determined by the non-parametric Kruskall–Wallis test. The contrasts between the different age points were tested with a *post hoc* test. Statistically significant differences are indicated.

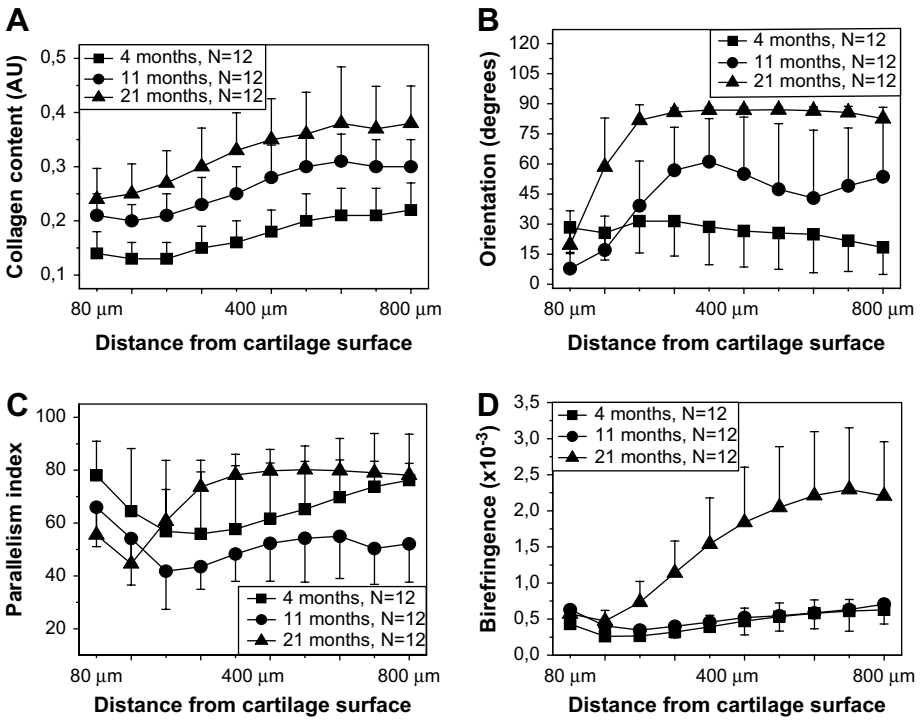

Fig. 6. Cartilage thickness decreased as the growth and maturation of animals proceeded. Therefore, microscopic analyses were also made by taking into account only the first 800 µm from the cartilage surface. This was close to the average thickness of the 21-month-old animals. The results show that remodelation is not explained by thinning of the cartilage. All measured parameters showed significant changes during growth and maturation. Values are given as mean ± S.D. Explanations for parameters and abbreviations are found in the text for Figs. 4 and 5.

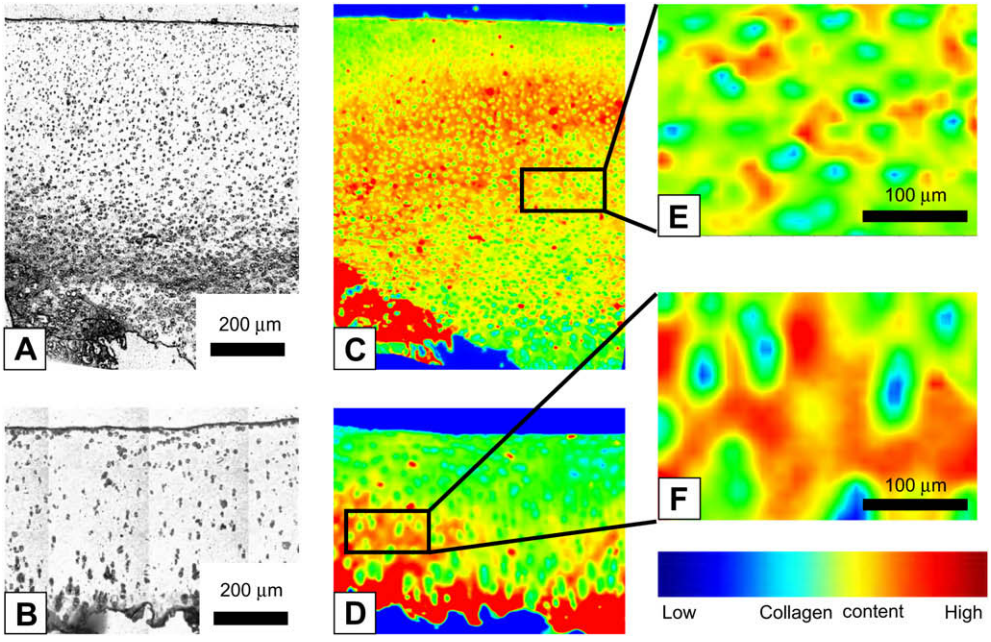

Fig. 7. Comparison of collagen content distribution in articular cartilage of 4-month-old (A, C and E) and 21-month-old pigs (B, D and F). Light microscopic images (A and B) reveal the cell lacunae as dark dots showing the dimensions of the lacunae. The FTIR image obtained using the amide I spectral wavelength indicates the collagen distribution in cartilage (C and D). The distribution of the collagen content reveals differences between the groups. The interterritorial cartilage matrix of mature animals (F) has a higher collagen content than that of the younger animals (E). Lacunae and the territorial matrix appear to contain less collagen than the interterritorial matrix.

Discussion

The results of the present study indicate that the collagen network of porcine articular cartilage undergoes significant remodeling during skeletal maturation. This was demonstrated with imaging techniques that are capable of visualizing architecture of the collagen fibril network and the spatial content of collagen. The 4-month-old pigs exhibited low collagen content and displayed a collagen fibril orientation parallel to the cartilage surface throughout the uncalcified tissue. This early fibril arrangement differs considerably from the classical Benninghoff model according to which the collagen fibrils run parallel to the surface in the superficial tissue and are orientated at right angles to the surface in the deep cartilage. The cartilage of the 11-month-old animals exhibited the largest variation in the orientation angles. Further, the parallelism index indicated substantial heterogeneity of the collagen network architecture in that group (Fig. 4). This structure seemed to represent a transitional stage between the immature cartilage phenotype present at a young age and the adult phenotype that was present at the age of 21 months. Birefringence was increased in the deep cartilage significantly at the age of 21 months. Birefringence could not detect the changes that occurred earlier, and virtually no difference was detected between the 4- and 11-month-old animals. Orientation and parallelism index seemed to be more sensitive indicators of the maturational changes than birefringence.

The initial collagen network of young animals was gradually transformed into a mature network with a novel architecture and increased collagen content. It seems reasonable to assume that this alteration reflects the tissue response to growth and maturation as well as altered joint loading conditions. The collagen content of cartilage increases significantly during maturation. At the same time, the distribution of collagen changes with tissue depth and also around the cell lacunae. These characteristics in tissue architecture and composition provide the foundation for the unique biomechanical properties of articular cartilage[34].

During growth, the conditions for rapid remodeling are probably ideal. The body weight and the muscular strength increase gradually over a lengthy time period. The immature collagen network is conditioned to achieve strength and the properties characteristic of the mature tissue. The collagen synthesis rate is highest at a young age[23]. As the maturation proceeds, the collagen synthesis rate reduces in parallel with a decrease in the matrix metalloproteinase levels. This probably also slows down the rate of cartilage remodeling.

A pig reaches puberty at the age of 6 months. Breeding of animals starts at 7 months of age. The youngest timepoint (4 months) would possibly correspond an age of 6–8 years in human life. Animals at the age of 11 months have passed puberty but the skeletal maturity is not yet reached (age possibly corresponds years 12–16 in humans). The porcine skeletal development continues surprisingly long and the growth cartilages close as late as at the age of 3–4 years of age (expected lifespan of pigs is 10–15 years)[36]. At 21 months, the musculoskeletal system of animals might correspond to the age of 20–25 years in humans. In the present study, there occurred a significant remodelation of the collagen network during skeletal development. These alterations possibly continue until the skeletal maturity is reached.

Our findings indicate that there is an extensive postnatal synthesis and reorganization at the collagen network. Tissue maturation requires time and the final phenotype of the tissue is only gradually achieved. The present results are basically in line with the ideas proposed by Gründer and Hunziker *et al.*[10,11]. However, Hunziker *et al.* claimed that the superficial zone of cartilage does not experience significant reorganization whereas the present findings indicate that significant alterations in the content, organization and orientation of the collagen fibrils also take place in the superficial zone of cartilage. Thus, the present results suggest that even the most superficial tissue is subjected to significant structural modifications.

Earlier results indicated that there was a high activity of matrix metalloproteinase enzymes in the articular cartilage during growth and adolescence[37–39]. It seems likely that both degradation and synthesis of collagen molecules and the assembly of new fibrils are required for the remodeling of the collagen network. We have observed a similar type of collagen network remodeling in other species, such as horse, cow and humans (unpublished observations). The present results are not specific to pigs alone. We suggest that different species undergo a similar type of maturation process in their articular cartilage, even though the activity and duration of cartilage maturation may vary. The age-dependent remodeling of articular cartilage should be borne in mind when experiments with pigs, or with other laboratory or domestic animals, are carried out. The age of the animals may significantly influence the results of experiments.

The functional properties of articular cartilage change as the collagen content and collagen fibril orientation mature. Previous studies on developing cartilage have related the biomechanical properties and chemical composition of tissue with each other[3,4,6]. In cartilage, PGs do not exhibit similar anisotropic properties as collagen, even though they are also non-homogeneously distributed. The collagen network significantly controls the mechanical parameters of cartilage, such as the tissue dynamic stiffness and the Poisson's ratio[40]. This is consistent with the concept that the collagen fibril arrangement governs the lateral expansion of the cartilage tissue during compression and highlights the importance of the orientation of the collagen fibrils when structure–function relationships of cartilage are characterized.

In summary, the present results indicate that the early postnatal collagen network of articular cartilage is gradually remodeled in growing pigs. The remodeling is controlled by the growth and maturation as well as, most probably, mechanical loading of the joints. In porcine articular cartilage, maturation is a slow process. Changes of the collagen network still continue at the age of 11 months. In the present study, the adult phenotype of cartilage tissue was observed by the age of 21 months. The timescale of tissue maturation for the human articular cartilage remains to be clarified. In principle, the growth and maturation period appears to represent a window of opportunity for undertaking musculoskeletal interventions intended to improve cartilage properties. Further evidence for the occurrence of exercise-related changes in cartilage was revealed by van Weeren *et al.*. In a controlled exercise study with horses these authors showed that musculoskeletal intervention at early age can modify the composition of the extracellular matrix of cartilage and influence the maturation process[41]. These findings might indicate that regular physical exercise at a young age may strengthen the cartilage collagen network and prevent, or at least postpone, the appearance of osteoarthritis later in life as previously speculated[21].

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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