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Simultaneous changes in bone mineral density and articular cartilage in a rabbit meniscectomy model of knee osteoarthrosis

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Summary

Objective: It was hypothesized that increased bone mineral density of the medial proximal tibia would precede or coincide with the development of more severe cartilage changes after meniscectomy.

Methods: In a rabbit knee model, mineral density of subchondral bone and changes of articular cartilage were monitored 13 to 40 weeks after medial meniscectomy or a sham operation.

Results: Both procedures resulted in a decrease of bone mineral density, especially of the medial proximal tibia, which persisted up to 40 weeks (P<0.02–0.0007). Meniscectomy induced cartilage changes typical for osteoarthrosis (P<0.009), which progressed over time on the posterior aspect of the medial tibial plateau (P<0.009), which is physiologically covered by the meniscus, but the procedure also induced iatrogenic changes which were located mainly on the anterior aspect of the concerned compartment, and which did not progress or develop to osteoarthrosis.

Conclusions: The data suggest that the cartilage changes after meniscectomy in this animal model are caused by the surgical trauma, subsequent limb misuse, and altered load distribution, and initially associated by a decrease not an increase in bone mineral density of the proximal tibia. Moreover, the cartilage changes progressed without a simultaneous increase of the bone mineral density at corresponding sites.

Key words: Rabbit knee, Meniscectomy, Bone mineral density, Osteoarthrosis.

Introduction

Development of osteoarthrosis of the knee joint after meniscectomy is common. Already after 3 months, discrete cartilage changes and formation of osteophytes were found after complete removal of the medial meniscus in the rabbit.[1] In clinical long-term follow-up studies joint space narrowing on radiographs indicating loss of cartilage is frequent after partial and total meniscectomy.[2,3] Bony spurs, flattening of the condyles and subchondral sclerosis, which were often noted at the same time, indicate that meniscectomy also induces conspicuous bone remodeling.

In an intact joint, bone density at the periphery of the proximal tibia underlying the meniscus is usually lower than at the central part not covered by the meniscus, but in joints with a lacking meniscus it was found increased at the periphery.[4] In a sheep model, the area of highest bone density in the proximal tibia had shifted to a more posterior and medial location one year after meniscectomy.[5] Bone mineral density in the proximal tibia was in general found increased 5 to 12 years after meniscectomy in man.[6,7] It is believed that this increase in bone mineral density reflects an adaptation to the changed loading situation of the knee joint after removal of the meniscus, especially the reduction of contact area and subsequent increase of peak stresses on the tibial plateau.[5,8,9] Compressive strains in the proximal tibia changed substantially after medial meniscectomy.[10] However, there are no reports about bone changes in the proximal tibia during the first months after meniscectomy. It seems therefore difficult to figure out whether the bone changes after meniscectomy precede the cartilage changes or vice versa, or whether they happen on the same time. Trabecular microfractures, which were induced by repetitive impact loading, healed with conspicuous callus formation, and resulted in stiffening of subchondral bone which preceded the cartilage changes.[11] Signs of stiffening were already observed 3 days after the first impact loading session.[12] It has been proposed that stiffening of subchondral bone may cause cartilage changes as a secondary phenomenon, and that progression of cartilage lesions requires stiffened subchondral bone.[13] In the situation after meniscectomy, the increase in bone mineral density of the proximal tibia may be associated to a stiffening of subchondral bone,[14,15] which eventually initiates or at least aggravates the cartilage changes after this procedure.

In the present experiment, 15 adolescent New Zealand white rabbits were operated with meniscectomy in one knee and a sham operation in the other with the purpose to quantify changes in cartilage morphology and subchondral

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bone density at various time intervals after operation up to 40 weeks. The hypothesis of the study was that a successive increase in subchondral bone mineral density, preferentially in the medial proximal tibia, would coincide or precede the development of more severe cartilage changes at this site.

Materials and methods

In 15 >4 month-old (range, 19 to 62 weeks) female virgin rabbits, weighing between 3.3 and 5.1 kg at time of surgery, the medial meniscus in the right knee was excised, and a sham operation was done in all left knees. Joint fluid was sampled before operation, every third week after operation until 12 weeks, thereafter every sixth week, and at death. The detailed procedure of joint fluid sampling and the results of this procedure are not presented in this paper. Equal numbers of rabbits ($N=5$) were killed after 13, 25 and 40 weeks. At death, all animals were fully grown (over 40 weeks old). Both knees from five normal adult female virgin rabbits (age range, 44 to 49 weeks; weight range 4.0–4.6 kg) were used as controls. The experiment was approved by the local ethics committee and the guidelines for care and use of animals were followed.

SURGICAL PROCEDURE

The operation was performed using sterile technique.[1] No premedication was used. After intravenous anesthesia with Ketamine (Parke-Davis, S.A., Barcelona, Spain) and Xylazinchloride (Bayer, Monheim, Germany) (15 mg/kg and 1.5 mg/kg, respectively), a vertical skin incision of 1 to 2 cm length was made at the site of the medial collateral ligament. The joint capsule was opened vertically both anterior and posterior to the latter. Firstly, the anterior insertional ligament of the medial meniscus was transected, and the anterior horn and middle part of the meniscus dissected free from its capsular attachment and the medial collateral ligament. The dissected part of the meniscus was then pushed under the medial collateral ligament to the posterior part of the joint. Its posterior insertional ligament was resected through the posterior capsular incision and the meniscus removed in one piece. Before wound closure, the joint was irrigated with physiological saline and a couple of drops of Oxytetracyclinehydrochloride (Pfizer, Amboise, France). The wound was closed in layers with 0–5 sutures. For sham operation, similar skin and capsular incisions were made but the meniscus left intact. At the end of both knee operations a single dose of antibiotics (Oxytetracyclinehydrochloride, 10 mg/kg) was injected intravenously. Postoperatively, the joint was not immobilized and free movement was allowed within the separate cages (0.5 m^2). The wound healing was assessed daily until full functional recovery. At indicated time, the animals were killed with an overdose Pentobarbitalnatrium (Apoteksbolaget, Umeå, Sweden).

Immediately after death, both hind limbs were disarticulated at the hip joint. The muscles were removed, but the knee joint capsule left intact. The femur was cut at the level of the trochanter major and the tibia above the ankle joint. The lower part of the fibula was removed from its synostosis to the tibia. The specimens were then wrapped in saline soaked gauze and kept refrigerated except for the radiographic evaluation and the bone mineral density measurement which was done at room temperature. The final preparation for histology was done within 36 hours after death. Radiographic examination showed that all animals had closed epiphyses.

MEASUREMENT OF BONE MINERAL DENSITY

Both knees of the operated animals and the right knees of each of the control animals were investigated. Bone mineral density of the regional high resolution option was measured by dual-energy X-ray absorptiometry (DEXA; hologic QDR 1000). Precision of the device was 1.0% in the spine and 1.3% in the femoral neck when measuring 30 patients and healthy subjects twice within a 2-week interval. The precision in measuring a spine phantom repeatly was 0.4% (coefficient of variation).[16] Each rabbit knee joint was positioned in maximal extension in a specially designed holder, and scanned with lateral and frontal projections. The scanning width was 2.1 cm, which included the entire knee joint width, and the length was 5 cm, which extended from the tibial insertion of the medial collateral ligament to approximately 1 to 1.5 cm above the femoral condyles (Fig. 1a,b) in both projections. In the five right control knees, the scanning procedure in both projections was repeated after complete unmounting.

HISTOLOGICAL EVALUATION

Immediately after the scanning procedure, both knees of operated and control animals were opened and dissected, and then processed for the histological evaluation. In the left control knees, which had not been scanned, the medial meniscus was removed in similar manner as during surgery, prior to dissection. This was done to see whether the procedure of meniscal removal did cause any immediate damage of cartilage. The gross appearance of articular cartilage and extent of resection of the meniscus were recorded on a protocol. All soft tissues around the knee were removed. The femur was cut above the trochlear groove and condyles, the tibial shaft below the tibial insertion of the medial collateral ligament. The femoral condyles and tibial plateaux were fixed separately in 4% paraformaldehyde solution for one week, and then decalcified in formic acid for 5 to 16 weeks. After decalcification, sagittal sections of 1 to 2 mm thickness were cut from the middle of the medial and lateral femoral condyles (Fig. 2a). The tibial plateau was firstly divided sagittally along its midline. Frontal sections of 1–2 mm thickness each were cut from the medial tibial plateau at anterior, central and posterior locations. From the lateral tibial plateau only a frontal section was taken from the central part (Fig. 2b). Each of the above-described six different specimens of one knee were embedded separately in paraffin, sections of 5-μm thickness were cut and stained with alcian-blue/periodic acid Schiff and safranin-O. Signs of osteoarthrosis, but also iatrogenic, man-made cartilage changes were evaluated by the validated modified Mankin score according to van der Sluijs.[17] The score describes changes in cartilage structure and matrix staining, and cellular abnormalities; it ranges from 0 (normal cartilage) to 13 (worst case of arthrosis) points. A minimum of five sections from different levels were used for evaluation of each tissue block. One experienced (KM, senior author) examiner and two less experienced examiners (BA, AF) evaluated independently all samples (interexaminer correlation: $r^2=0.41–0.50$; $P<0.01$). For further analysis, the values from the experienced examiner (KM) were used.

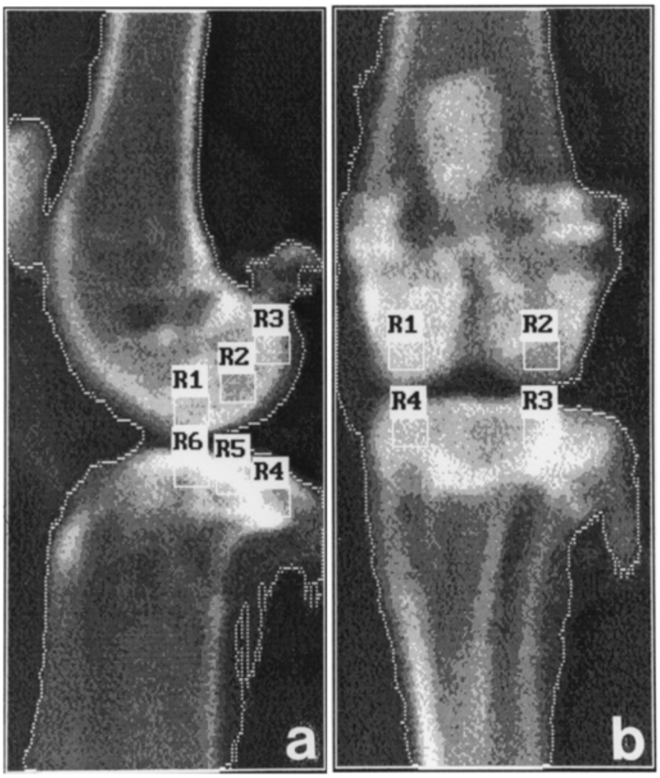

Fig. 1. (a) Lateral projection of the rabbit knee during the scanning procedure. Squares R1 and R6 were marked in the subchondral area of the femoral condyle and tibial plateau respectively at their point of contact during full extension. Squares R3 and R4 were placed subchondrally under the apex of the posterior femoral curvature and the most posterior part of the tibia at the point of femorotibial contact during full flexion, respectively. Squares R2 and R5 were posed between squares R1,3 and R4,6, respectively. (b) Frontal projection of the rabbit knee during the scanning procedure. The squares were put subchondrally approximately 1 mm below the joint line at the areas of contact between the femoral condyles and the tibial plateaux. R1, medial femoral condyle; R2, lateral femoral condyle; R3, proximal lateral tibia; R4, proximal medial tibial.

CALCULATIONS

Bone mineral density measurement

On the lateral projection, six areas (lateral, 1–6) of 19×9 pixels corresponding to 0.0549 cm^2 were chosen, on the frontal projections four areas (frontal, 1–4) of similar size were marked (Fig. 1a,b). The squares of the lateral locations 1 and 6 were marked in the subchondral area of the femoral condyle and tibial plateau respectively at their point of contact during full extension. The square of the lateral locations 3 and 4 were placed subchondrally under the apex of the posterior femoral curvature and the most posterior part of the tibia at the point of femorotibial contact during full flexion, respectively. The squares of the remaining two locations on the lateral projection (2 and 5) were posed between squares 1,3 and 4,6, respectively (Fig. 1a). On the frontal projection, the squares were put subchondrally approximately 1mm below the joint line on the graph at the areas of contact between the femoral condyles and the tibial plateaux (Fig. 1b). The bone mineral density (bone mineral concentration adjusted by measurement area; g/cm^2) was used for further calculations.

All 10 defined areas from lateral and frontal projections were repeatedly measured on identical scans of both knees from 11 animals: (1) immediately after one measurement, (2) 2 hours after the measurement, and (3) again 3 months after the measurement. In all control knees (N=5), all measurements were done on the two different scans from each knee.

HISTOLOGICAL EVALUATION

The cartilage was scored separately at anterior and posterior parts of the medial and lateral femoral condyles (Fig. 3a). The anterior part corresponded roughly to the area where the femoral condyles and tibial plateau are in contact during extension of the knee joint, the posterior part corresponded to the posterior curvature of the femoral condyles, where the knee joint surfaces are in contact during knee flexion. On tibial sections, the central part corresponding to the area uncovered by the meniscus was scored separately from that of the peripheral one which is covered physiologically by the meniscus (Fig. 3b). A combined score for the medial and lateral femoral condyles, respectively, was formed by adding the values of the anterior and posterior regions and dividing them by 2. Also for the medial and lateral tibial plateaux, a combined score was calculated. For the medial tibial plateau all six values were added from anterior, central and posterior samples and divided by 6, for the lateral one the values of the central and peripheral parts were added and divided by 2. In addition, the values from the central part of the three samples from the medial tibial plateau were added and divided by 3, representing the score of the central part

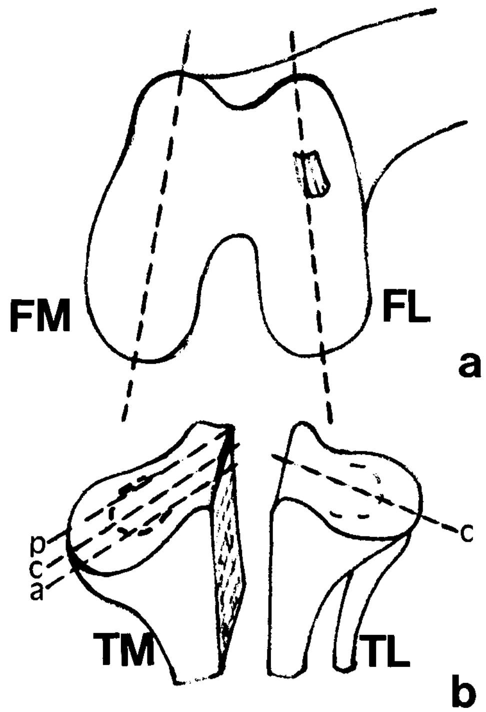

Fig. 2. Six different sites of the rabbit knee were evaluated histologically, which are represented by straight lines. On the femur, two sites were evaluated (a), on the tibia four sites (b). FM, medial femoral condyle; FL, lateral femoral condyle; TM, medial tibial plateau; TL, lateral tibial plateau; p,c,a, posterior, central and anterior locations on the tibial plateau, respectively.

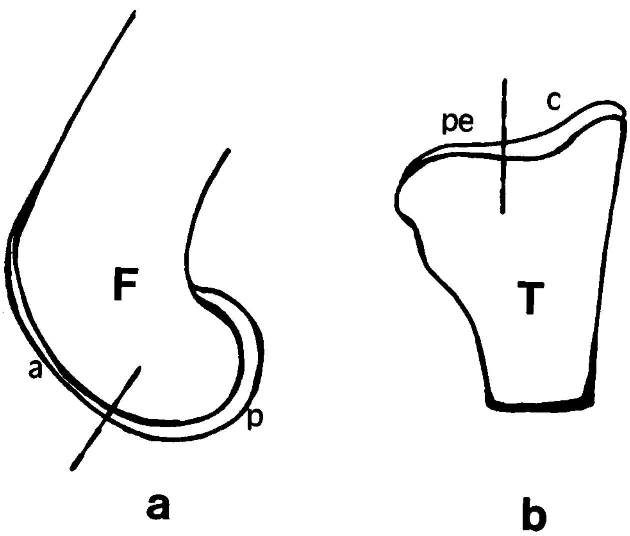

Fig. 3. The sections were scored separately at anterior (a) and posterior (p) locations on the femoral samples (a) and on central (c) and peripheral (pe) locations on the tibial samples (b). F, femoral section, T, tibial section.

of the medial tibial plateau which is physiologically not covered by the meniscus. The same was done for the three peripheral samples, representing the part of the tibial plateau which is covered physiologically by the meniscus. Further, an overall score was formed for each joint by adding the combined values of the medial and lateral femoral condyles and tibial plateaux, and dividing them by 4. Finally, the highests degree of arthrosis found in one joint was used to express the maximum degree of arthrosis of an individual joint.

STATISTICS

Test-retest correlation between repeated measurements of same or repeated scans, or correlations between age of the animal, duration of follow up, and bone density values were calculated with tests for linear correlation. For correlations between the histological scoring from different examiners, or correlations between age of the animals, duration of follow up and histological scores, the Spearman rank correlation for non-parametric values was chosen on a 5% significance level. For paired comparisons between the operated and non-operated knees, the Student's paired *t*-test was used for parametric variables (bone mineral density values) and the Wilcoxon signed rank test for nonparametric data (histological scores). A significance level of $P<0.05$ was required. Differences between knees from different animals were calculated by unpaired testing using analysis of variance and post hoc tests for planned comparisons for parametric variables on a 2% significance level ($P<0.02$). Kruskal Wallis analysis of variance and Mann-Whitney U-tests were used for calculation of non-parametric variables (histological scores). To compensate for the increasing probability of type-I error during multiple comparisons of non-parametric value, which is not automatically included in the Kruskal Wallis analysis of variance, the *P*-level was adjusted in the analysis of nonparametric variables. A significance level of $P<0.01$ was required. Multivariate analysis of variance (MANOVA) was used to determine the overall effects of location, choice of surgical method and time on bone mineral density. All calculations were performed using commercially available software (STATISTICA, Statsoft Inc®, Tulsa, OK)

Results

Two rabbits were replaced because of accidental death during anaesthesia.

BONE MINERAL DENSITY

Test–retest correlations between repeated calculations of identical scans following immediately each other were highly significant (mean r^2=0.98) for each single location. Repeated calculations after 2 hours or 3 months were lower but still highly significant (mean r^2=0.64). Repeated scanning and calculations after unmounting and subsequent relocation of the knee specimen had a test-retest correlation of r^2=0.74. The mean difference between the two different scans of one knee was 0.03 g/cm^2 and the 95% confidence interval ±0.04 g/cm^2.

There was no correlation between bone mineral density and age at operation, age at death or duration of follow-up time after surgery.

In general, bone mineral density was higher on lateral than frontal projections (MANOVA, $P<0.0001$). Choice of surgical method (meniscectomy, sham operation) had no overall effect on bone mineral density. At individual analysis, there was no difference in bone mineral density between sham-operated and meniscectomized knees except at a single location on frontal projection (medial femoral condyle—region FR1) at a single time point (knees

Table I
Bone density/standard deviation (g/cm^2)

Group	LR1	LR2	LR3	LR4	LR5	LR6	FR1	FR2	FR3	FR4
ME 13	0.7447	0.6432	0.6114	0.8233	0.7910	0.9029	0.2049	0.2481	0.2131	0.0825
	/0.1025	/0.1072	/0.1738	/0.1967	/0.2398	/0.1090	/0.0629*‡	/0.0258	/0.0586*	/0.0474*
ME 25	0.7292	0.5430	0.6451	0.7827	0.8158	0.8473	0.4050	0.2946	0.3957	0.2155
	/0.1570	/0.1983	/0.2176	/0.3159	/0.2469	/0.1958	/0.1677	/0.1724	/0.1918	/0.2315*
ME 40	0.8880	0.7064	0.6539	0.8564	0.9768	1.0102	0.2504	0.3220	0.2851	0.1047
	/0.0896	/0.0884	/0.1710	/0.1583	/0.1842	/0.1320	/0.0607*	/0.0624	/0.0891*	/0.0707*
Sh 13	0.6962	0.6226	0.6740	0.9444	0.8745	0.8398	0.3193	0.2889	0.3636	0.2080
	/0.1078	/0.1354	/0.1609	/0.1969	/0.1042	/0.0817	/0.0840*	/0.1871	/0.2687	/0.2322
Sh 25	0.7591	0.7435	0.8823	1.0425	0.9977	0.9480	0.3003	0.2137	0.2166	0.1517
	/0.0896	/0.0780	/0.1457	/0.2545	/0.1325	/0.1190	/0.0832*	/0.0314	/0.0710	/0.1119
Sh 40	0.8005	0.7217	0.7100	0.7590	0.8749	0.8893	0.3444	0.3075	0.4229	0.2305
	/0.0771	/0.0830	0.1997	/0.1999	/0.1395	0.1688	/0.0759*	/0.1638	/0.2997	/0.2386
C	0.7134	0.5932	0.7015	0.9415	0.8511	0.8584	0.5273	0.5035	0.5804	0.4988
	/0.1729	/0.1970	/0.2130	0.2280	/0.1554	/0.1426	/0.1551	/0.2176	0.2733	/0.2281

ME, meniscectomy; Sh, sham operation; C, control; 13,25,40 follow up time in weeks; L, lateral projection; F, frontal projection; R, region; 1,2 . . ., different scanned areas, see Figs 2a,b.

*Significant difference compared to same area in control group (ANOVA and post-hoc tests), *P*<0.02–0.0007; ‡significant difference compared to left sham-operated knee (paired comparisons), *P*<0.02.

with 13-week follow up). Here meniscectomized knees had a lesser bone mineral density than at the corresponding area in the opposite sham-operated knee (*P*<0.02) (Table I). There were no differences in bone mineral density between control and experimental animals on lateral projections. On frontal projections, operated knee joints regardless of type of initial treatment (meniscectomy, sham operation) had in general a lower bone mineral density at all four measured locations than the controls (MANOVA, *P*<0.00001) especially at the medial compartment in the short- and long-term groups (Table I). Bone mineral density values of medial and lateral femoral condyles within individual knees were similar in all groups. Control animals had also similar density values at the lateral and medial tibial plateau within one joint. In contrast, the lateral tibial plateau had in general higher values than the corresponding medial one in sham-operated (*P*<0.002) and meniscectomized knees (*P*<0.000009), when the values of all experimental animals were compared.

GROSS APPEARANCE

In all meniscectomized knee, the medial meniscus had been excised completely except for an occasional remnant of the posterior horn and its attachment ligament. Significant regrowth of the excised meniscus was not noted. The tibial cartilage in all meniscectomized knees showed fibrillation, and all except one had osteophyte formation at the periphery of the plateau. Also most of the medial femoral condyles showed cartilage fibrillation (*N*=9/15) and in some cases an osteophyte had developed (*N*=5/15). The lateral compartment did not show any macroscopic changes. Four sham-operated knees had some cartilage fibrillation on the medial tibial plateau and three of them a tibial osteophyte. There were no other changes in this group.

HISTOLOGY

There were no signs that the storage time before the histological fixation had adversely influenced the quality of the cartilage. Surgery was associated with a high risk for iatrogenic damage to articular cartilage. In 13 and 11 of the 15 experimental knees in each group (meniscectomy, sham operation), respectively, there were signs (cuts) that the surgical approach or joint fluid sampling had injured articular cartilage. This was most frequent on the anterior part of the medial femoral condyle (*N*=18) (Fig. 4a). Occasionally, the lateral femoral condyle, medial or lateral tibial plateau showed some cuts, preferentially at anterior or central locations. Most knees had these changes at a single location (*N*=17), and some at two locations (*N*=7). The iatrogenic character of these injuries was confirmed by histological evaluations of the controls, in which the meniscus had been removed post mortem by a similar approach as during operation (Fig. 4b). Here, cuts into articular cartilage were mainly noted on the anterior aspect of the femoral condyles or central aspect of the tibial plateau. These iatrogenic, man-made injuries did nevertheless not show a major tendency to result in osteoarthrosis of the concerned area, as the reaction of the adjacent cartilage matrix was only little even as long as 25 or 40 weeks after operation (Fig. 4a).

There was no correlation between age at operation or death, duration of follow up and the combined histological score in meniscectomized or sham-operated knees. A significant negative correlation existed between age at operation and the peripheral region of the central section of the medial tibial plateau in meniscectomized knees (TMc-pe, $r^2=-0.41$, *P*<0.009). In addition, there was a highly significant positive correlation between duration of follow up and the histological score of the peripheral cartilage of the posterior section of the medial tibial plateau in the same group (TMp-pe, $r^2=0.66$, *P*<0.0002). All other investigated joint areas after meniscectomy or sham-operation did not correlate to the age of the animals at either operation or death, or duration of follow up.

Group differences

All meniscectomized groups had a higher degree of cartilage changes on the posterior region of the medial femoral condyle (FMp, Table II) than the controls

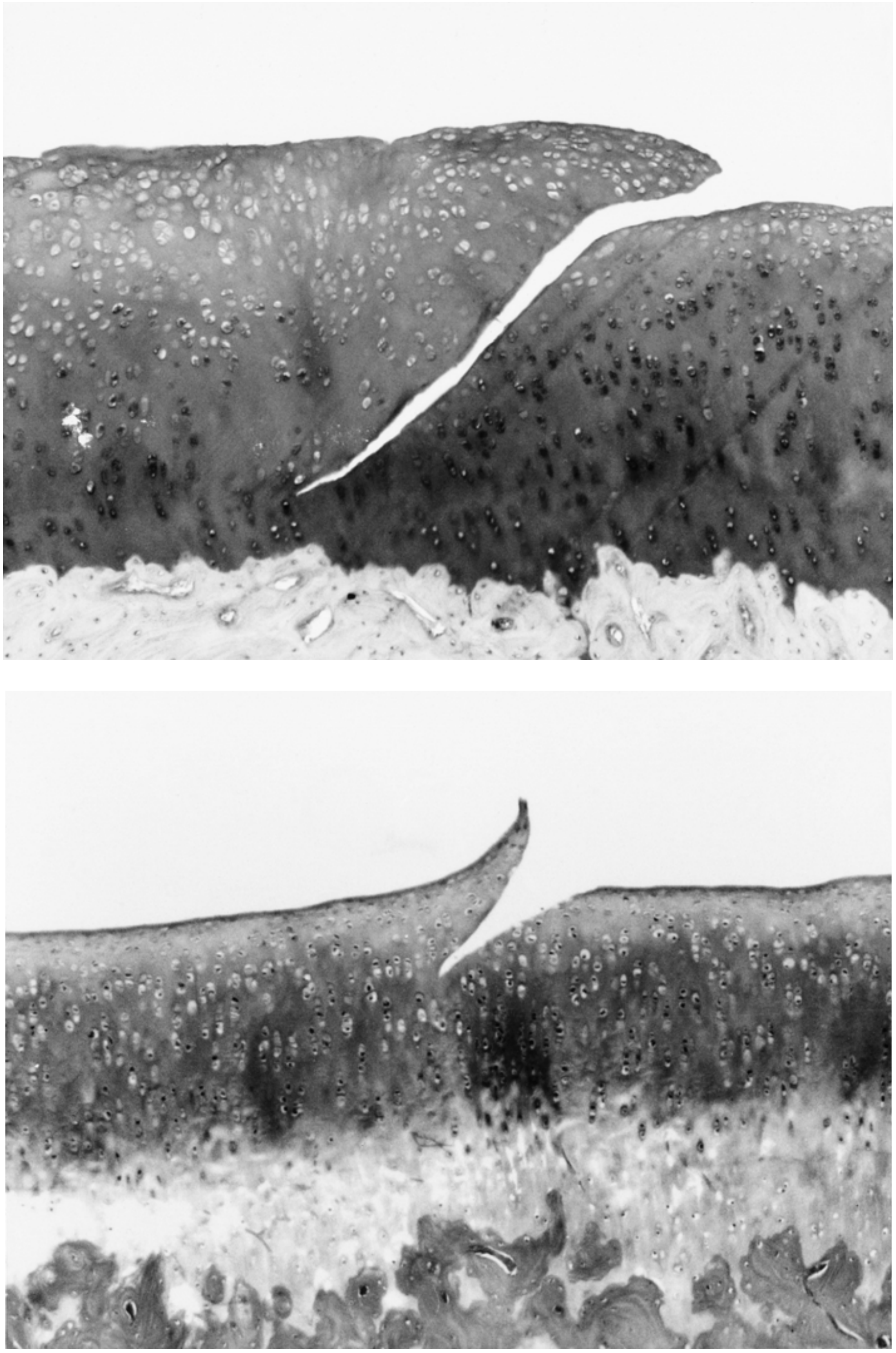

Fig. 4. (a) Histological sample of the medial femoral condyle (anterior location) 24 weeks after meniscectomy, showing a iatrogenic, man-made cut. The wound edges are less cellular than the remaining cartilage matrix, but otherwise there is little reaction (AB-PAS, ×200). (b) Histological sample of the medial femoral condyle (anterior location) of a control animal immediately after meniscectomy (Safranin-O, ×100).

(P<0.009), but there were no overall differences between the follow-up times at this region (Fig. 5a). In all meniscectomized groups, the cartilage on the peripheral location of the posterior section of the medial tibial plateau (TMp-pe, Table II), which is physiologically covered by the meniscus, had a higher degree of changes than in controls (P<0.01). In addition, cartilage changes were more conspicuous in animals with a 40-week follow up than in animals with 13-week follow up (P<0.009, Table II) (Fig. 5b).

In contrast, sham-operated knees usually showed no significant site-specific difference to controls except for a single location in a single group: animals with a 40-week follow up had more cartilage changes on the central section of the medial tibial plateau (central location, TMc-c, Table II) than controls (P<0.009).

Table II
Histological scores at specific locations (range)

Group	FM		FL		TMa		TMc		TMp		TLc	
	a	p	a	p	c	pe	c	pe	c	pe	c	pe
ME 13	3 (1–12)	3*‡ (1–5)	1 (0–5)	0 (0–1)	1 (0–4)	1 (0–7)	3 (0–6)	2 (0–3)	5‡ (3–8)	5*‡ (2–6)	0 (0–0)	0 (0–5)
ME 25	4 (1–11)	5*‡ (1–9)	0 (0–1)	0 (0–1)	3 (0–8)	4 (0–10)	3 (1–9)	3 (0–5)	4 (1–13)	6*‡ (4–7)	0 (0–0)	0 (0–1)
ME 40	2 (0–6)	6*‡ (2–13)	1 (0–4)	0 (0–3)	1.5 (1–3)	1.5 (0–3)	3 (1–4)	3 (1–5)	3 (2–4)	7*‡§ (7–13)	0 (0–0)	0 (0–0)
Sh 13	0 (0–8)	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–1)	1 (0–4)	1 (0–6)	0 (0–3)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–0)
Sh 25	1 (0–9)	0 (0–0)	0 (0–5)	0 (0–2)	0 (0–3)	0 (0–3)	3 (0–9)	0 (0–2)	0 (0–4)	0 (0–0)	0 (0–6)	1 (0–3)
Sh 40	0 (0–9)	0 (0–3)	0 (0–1)	0 (0–3)	0.5 (0–1)	0 (0–1)	4* (1–12)	1 (0–3)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)
C right	0 (0–5)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–1)	0.5 (0–5)	0 (0–1)	0 (0–0)	0 (0–0)
C left	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–1)

ME, meniscectomy; Sh, sham operation; C, control; 13,25,40, follow up time in weeks; FM, medial femoral condyle; FL, lateral femoral condyle; TMa, anterior medial tibial plateau; TMc, central medial tibial plateau; TMp, posterior medial tibial plateau; TLc; central lateral tibial plateau; a, p, c, pe, anterior, posterior, central or peripheral location on histological sample (see Figs 3a,b).

*Higher scores than controls ($P<0.01$); ‡higher scores than in matched sham-operated joints of same time interval ($P<0.04$); §meniscectomized knees at 40 weeks had higher scores than at 13 weeks.

All three meniscectomized groups had higher combined scores of the medial tibial plateau than controls (TM, Table III, $P<0.01$). This was also true, when the combined scores of the central and peripheral part of the medial tibial plateau were counted separately (TMC, TMPe; $P<0.01$). Also the combined score for the whole joint and the degree of maximum arthrosis was higher in meniscectomized knees than in controls ($P<0.01$). However, there were no significant differences between the meniscectomized groups.

Only sham-operated knees with longer follow up (25 or 40 weeks) had higher whole joint arthrosis scores or maximum scores compared to controls ($P<0.01$). There were no differences between the sham-operated groups.

Right and left knee differences

There was no difference between right and left control knees. Significant overall differences between right and left knees of experimental animals ($N=15$) were found on the posterior location of the medial femoral condyle (FMp), on the central locations of the anterior and posterior sections of the medial tibial plateau (TMa-c, TMp-c), and on the peripheral locations of the central and posterior sections of the medial tibial plateau (TMc-pe, TMp-pe, Table II) ($P<0.01$). In all instances meniscectomized knees had more cartilage changes than sham-operated knees. Overall, the combined scores of the medial femoral condyle and medial tibial plateau, as well as the combined scores of the central and peripheral part of the medial tibial plateau were higher in meniscectomized than sham-operated knees (FM,TM,TMC,TMPe, Table III) ($P<0.01$) ($N=15$). The same was true for the combined score of the whole joint and the maximum arthrosis score (All, Max, Table III). Significant differences between the five paired meniscectomized and sham-operated knees at the three different follow-up evaluations were fewer than when the results from all 15 animals were combined (Tables II, III).

Discussion

The intra-articular operation, regardless of type of procedure (meniscectomy, sham operation), resulted in a decrease in bone mineral density on frontal projections, which was most pronounced in the proximal medial tibia. This effect remained over a period of 40 weeks after the procedure. On the other hand, the fact that there was no reduction in bone mineral density on lateral projections indicates that the change was only minor.

Beside the changed loading situation after meniscectomy, limb misuse thanks to pain after the surgical procedure, repeated joint fluid sampling, and hemarthrosis may have caused this bone remodeling with resulted in a decrease in bone mineral density. Limb misuse after either surgical procedure was probably the main factor for the demineralization and may explain why there was no major difference between meniscectomy and a sham operation. In man, rupture of the anterior cruciate ligament induced permanent osteoporosis in the concerned knee.[18] Decreased bone mineral density of the distal femur was also noted in patients with meniscal symptoms already before surgery, and was found to persist up to 5 years after meniscectomy though the symptoms had subsided.[19] In contrast, bone at the proximal tibia of the concerned compartment showed an increase of bone mineral density 5 to 12 years after clinical meniscectomy,[6,7] most probably reflecting a long-term adaptation to the increased local stresses after this procedure. In the rabbit, meniscectomy and the subsequent stress increase on the tibial plateau had up to 40 weeks apparently not yet counteracted the decrease in bone mineral density at this site due to the operation and general limb misuse.

After medial meniscectomy or a sham operation, bone mineral density was lower on the medial than lateral proximal tibia unlike in the controls, which had equal values on both sites. This may indicate that the medial tibial plateau was rather deprivated from high stresses opposite

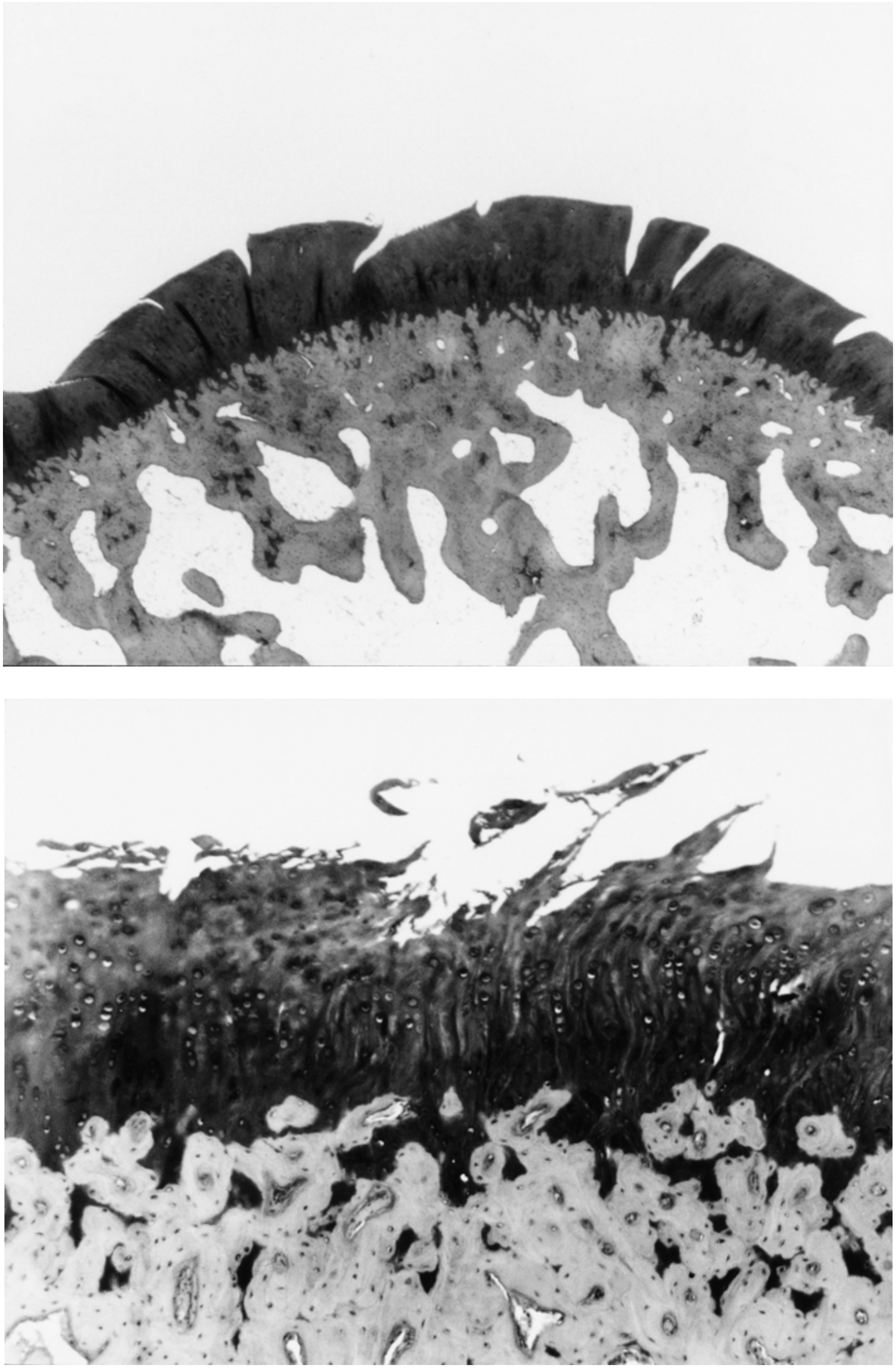

Fig. 5. (a) Histological sample of the medial femoral condyle (posterior location) 40 weeks after meniscectomy. The cartilage shows multiple clefts down to the calcified zone, which is typical for osteoarthrosis (AB-PAS, ×25). (b) Histological sample of the medial tibial plateau (peripheral location of a section from the posterior part of the plateau, TMp-pe) 40 weeks after meniscectomy showing severe osteoarthrotic changes with complete matrix derangement (AB-PAS, ×100).

to what one would expect after a medial meniscectomy. Since demineralization was common in the medial compartment even after an arthrotomy (sham operation), it may be suggested that the animal rather loaded the lateral compartment of the knee, to avoid pain sensation. If the medial compartment is not enough loaded the femoral and tibial condyles will hardly contact when the meniscus is absent,[20] and the stresses on the tibia will be low.

Nevertheless, even if the medial compartment is loaded properly, it has been shown that the immediate effect of meniscectomy on joint contact areas and stresses is soon diminished (but not normalized) by adaptation of the condyles which takes place after meniscectomy *in vivo*.[21] Osteophyte formation, as frequently found here, is a form of adaptation, softening of damaged cartilage another.[22] There are no data from clinical material with similar

Table III
Combined histological scores and maximum score (range)

Group	FM	FL	TM	TL	TMC	TMPe	All	Max
ME 13	3 (2–7.5)	0.5‡ (0.5–2.5)	3‡* (3–3)	0 (0–2.5)	3.5*‡ (1.5–3.5)	2.5*‡ (2–4)	2*‡ (1.5–3.5)	8* (6–12)
ME 25	4.5 (3–7.5)	0 (0–1)	4* (2–6.5)	0 (0–0.5)	5* (1.5–6.5)	4.5*‡ (2.5–6.5)	2.5* (1.5–3.5)	10* (7–13)
ME 40	4.5 (2–7.5)	1 (0–3.5)	3*‡ (2.5–4.5)	0 (0–0)	2.5* (2–3.5)	4*‡ (3–6.5)	2.5*‡ (1.5–3)	8* (7–13)
Sh 13	0 (0–4)	0 (0–0.5)	1 (0–1.5)	0 (0–0)	0.5 (0–2)	1.5 (0–1.5)	0.5 (0–1.5)	4 (0–8)
Sh 25	0.5 (0–4.5)	0 (0–3.5)	0.5 (0–2)	0.5 (0–4.5)	1 (0–3.5)	0.5 (0–1)	1.5* (0.5–2)	5* (2–9)
Sh 40	0.5 (0–6)	0 (0–2)	1* (1–2)	0 (0–0)	1.5* (1–4)	0.5 (0–1.5)	0.5* (0–2.5)	4* (3–12)
C right	0 (0–2.5)	0 (0–0)	0 (0–1)	0 (0–0)	0.5 (0–1.5)	0 (0–0.5)	0 (0–0.5)	1 (0–5)
C left	0 (0–0)	0 (0–0)	0.5 (0–0.5)	0 (0–1)	0.5 (0–0.5)	0.5 (0–0.5)	0 (0–0.5)	1 (1–2)

ME, meniscectomy; Sh, sham operation; C, control; 13,25,40, follow-up time in weeks; FM, combined score of medial femoral condyle; FL, combined score of lateral femoral condyle; TM, combined score of medial tibial plateau; TL, combined score of lateral tibial plateau; TMC, combined score of central portion of medial tibial plateau; TMPe, combined score of peripheral portion of medial tibial plateau; All, combined score of whole joint; Max, maximum degree of cartilage change in an individual joint. *Higher scores than controls at specific time point (P<0.01); ‡higher scores than paired sham-operated knees at specific time point (P<0.04).

short-term follow up of tibial bone. It may also be argued that the bone remodeling process in the proximal medial tibia which finally leads to increased mineralization after meniscectomy was not yet finished at 40 weeks in the rabbit, and that increased mineralization will eventually occur later.

Hemarthrosis and unweighting have shown to initiate cartilage changes, especially when the conditions are prolonged.[23,24] In accordance, cartilage changes were common in this rabbit model both after meniscectomy and a sham operation, though the changes were less pronounced after the latter procedure. Part of them could be attributed to the surgical trauma and iatrogenic cartilage changes, which was confirmed by the changes seen immediately after meniscectomy, and part of them were probably caused by the altered load transfer between femur and tibia in the medial compartment owing to the surgical trauma and a lacking meniscus. The latter changes were found on the posterior area of the femoral and tibial condyles, the contact area of the knee during maximal flexion, the position which a caged rabbit preferentially uses. These changes were typical for osteoarthrosis with fibrillation, cleft formation up to complete derangement of the matrix, and they progressed with time on the medial tibial plateau after meniscectomy.

Conversely, the iatrogenic, man-made changes, which were found mainly on the anterior part of the joint showed no progression, and did not resemble osteoarthrotic changes as long as after 40 weeks. Here, the main reaction was hypocellularity and chondrocyte clustering near to margin of the cut, and locally decreased stain for proteoglycans similar to changes found after superficial cartilage laceration.[25] Though these iatrogenic changes were more frequently found at locations, where osteoarthrosis was uncommon as shown by the cases which were evaluated immediately after meniscectomy, it can not be excluded that iatrogenic changes had in some instances initiated the development of osteoarthrosis. Especially in cases with advanced osteoarthrosis it is difficult to differentiate whether there had been iatrogenic changes in the bottom of the lesion. In meniscectomized knees, gait impairment, hemarthrosis and a lacking meniscus may have had an additive effect and explain why these knees had a higher degree of osteoarthrosis than the sham-operated knees. This was confirmed by the fact that the cartilage changes and their progression with time were most common at the peripheral aspect of the tibial plateau which is physiologically covered by the meniscus.

The significant inverse correlation between age of the animal at operation and development of cartilage changes, which was found on the peripheral area of the medial tibial plateau after meniscectomy may be explained by the higher sensitivity of young articular cartilage to inflammatory agents compared to adult cartilage.[26] The association between age at operation and development of cartilage changes was otherwise uncommon in this material, probably since most of the animals were adult at time of operation.

Interestingly, the cartilage changes after meniscectomy were not associated to an increase in bone mineral density of the proximal medial tibia or subchondral sclerosis as shown in clinical investigations.[2,6,7] The condition of knee osteoarthrosis has been found associated with an increased bone mineral content of the proximal tibia.[27] On the contrary, cartilage changes in this rabbit meniscectomy model did progress despite a bone mineral density which was below normal. It can not be answered whether the decrease in bone mineral density was the cause of the cartilage changes or vice versa or whether these two features just coincided. In any case, the explanation that cartilage changes may occur secondary to bone mineral density increase and hardening of tibial subchondral bone does apparently not apply for the early time after meniscectomy in the rabbit. The former theory was established in an animal model using impact loading.[11] In the rabbit meniscectomy model the surgical trauma with subsequent limb misuse and an altered load distribution on cartilage itself rather than an increase in bone mineral density have to be discussed as initial causes for the cartilage changes.

Before the final histological preparation the knee joint specimens were stored for maximum 36 hours in order to enable various evaluations. Though such a prolonged storage time incorporates a risk for cartilage damage thanks to dehydration and proteolysis of the tissue we did not note a decrease in cartilage quality at the histological evaluation compared to cartilage specimens which are prepared immediately after death (own observations). Cartilage damage was probably limited owing to an intact joint capsule and storage in cold environment (4–8°C) for most of the time. Further, histological evaluation at low magnification may not be sensitive enough to pick up a minor decrease in tissue quality. Hence, it can not be excluded that the cartilage had suffered from the prolonged storage time. Nevertheless this should not have a major influence on the conclusions of this study, since the same storage conditions applied for all specimens and most results are based on comparisons between the groups.

The function and configuration of the rabbit knee joint, as well as the tissue properties of lapine articular cartilage and subchondral bone certainly differs from that in man. It is therefore difficult to assess whether the changes found here also apply for the situation of a patient with meniscectomy. Only clinical investigations with early assessment of bone mineral density after meniscectomy can answer this question.

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