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The groove model of osteoarthritis applied to the ovine fetlock joint

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Summary

Objective: Until now there have been no appropriate models for metacarpophalangeal osteoarthritis (OA), even though OA in this joint is a significant medical and economic problem in horses. A good model would be useful to evaluate progression and treatment of OA, particularly in this joint. Therefore, we translated the canine Groove model to the ovine metacarpophalangeal (fetlock) joint.

Method: Cartilage surfaces of the metacarpal side of one fetlock joint were surgically damaged (grooved), followed by intermittent forced loading of the experimental joint. After 15 and 37 weeks, cartilage, synovial tissue and subchondral bone were analyzed by the use of macroscopy, histology, biochemistry and micro-CT.

Results: Technically, the model was difficult to use because cartilage surfaces were very thin. Nonetheless, all macroscopic, histologic, and biochemical cartilage parameters demonstrated adverse changes in chondrocyte activity and matrix integrity. Decreased proteoglycan content suggested slow progression of cartilage degeneration over time, while synovial inflammation diminished. Impaired subchondral bone quality and osteophyte formation were found. Although osteophyte formation was progressive, subchondral bone changes diminished over time.

Conclusion: The canine Groove model appears to a limited extent transferable to the ovine fetlock joint. However, despite development of adverse changes consistent with early changes of OA, use of the Groove model in the ovine fetlock joint has technical limitations. Using larger animals, such as horses, may significantly improve the technical procedures and with that may provide a more reliable model of metacarpophalangeal OA that is based primarily on intrinsic cartilage damage, appropriate to evaluate the progression and treatment of OA in this particular joint.

Key words: Animal model, Sheep, Cartilage, Osteoarthritis.

Introduction

Osteoarthritis (OA) in humans is a common disease of joints, affecting over 10% of the adult population[1]. Musculoskeletal disorders, including OA, are also a leading cause of morbidity in veterinary medicine, specifically in horses. Research in breeds of racehorses found that 85% of injuries are musculoskeletal in nature[2]. About nine tenths of those injuries are in forelimbs[3]. One of the joints in horses most frequently affected by arthritis is the metacarpophalangeal (fetlock) joint[4]. Similarly to humans, these injuries can be related to excessive joint motion while exercising on uneven ground, excessive training, poor anatomy, or improper shoeing[4]. Hardly any models of OA are available to study progression and treatment modalities in the equine forelimb metacarpophalangeal joint[4].

Recently, a new canine model of joint degeneration with features as observed in early OA (the 'Groove' model), has been described in literature[5–7]. In this canine model, damage to the articular cartilage of the weight-bearing areas of the femoral condyles in the knee is the trigger for development of joint changes consistent with early OA. To increase this trigger, loading of the affected joint is forced/intensified by fixing the contra-lateral control limb to the trunk of the dog intermittently (2 h for 3 days a week). Biochemical and histological evaluations demonstrate degenerative changes in the joint, which closely resemble those in the anterior cruciate ligament transection (ACLT) model, one of the most frequently used (canine) models of (knee) OA[5,6].

In the canine Groove model at 10 weeks, collagen is damaged and proteoglycan (PG) turnover is disturbed; an ineffective synthesis combined with an enhanced release results in diminished PG content. Matrix metalloproteinase (MMP) activity measured in synovial fluid is enhanced. Histologically, moderate cartilage destruction, characterized by loss of safranin-O staining, fibrillation of the articular surface and chondrocyte clustering are evident. These characteristics of OA are also clearly visible in the tibial plateau, although this cartilage surface is not harmed during surgery. These types of changes are very consistent between animals. Most importantly, only mild signs of inflammation are present as demonstrated histologically. Furthermore, the degenerative changes in the cartilage matrix integrity

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slowly progress over time in the first year after induction, while synovial inflammation decreases[5]. Whether the slowly progressive phase in the first year after the initial induction phase will proceed into full-blown OA in several years, as demonstrated with the ACLT model[8], remains to be proven. But importantly, the observed characteristics of joint degeneration in the Groove model are not just the expression of the surgically applied damage but are the results of progressive features of (experimental) joint damage, because 3 weeks after surgery no features of progressive joint degeneration are present. Rather, repair activity at that time point is still observed[7] which at prolonged time points (10, 20, and 40 weeks) appears to fail[5,6]. Because joint damage in this model depends on intrinsic cartilage damage and not on joint instability or other permanently induced changes, the model might be sensitive to chondroprotective treatment and cure of the disease might even be possible[5].

Although many animal models of OA have been developed, there are not many equine models of OA, especially not with respect to the fetlock joint[4], a joint of major medical and economic interest in equine veterinary practice. Therefore, it was considered worthwhile trying to develop a model of OA for the forelimb metacarpophalangeal (fetlock) joint with the same characteristics as the Groove model. The limited accessibility of horses for experimental studies and ethical and financial reasons make it difficult to evaluate such a new model directly in horses. Therefore, first it had to be determined whether it is possible to translate the Groove model originally designed for the canine knee joint to the fetlock joints of sheep. This way could lead to the development of a screening model for treatment modalities directed against the intrinsic damage in articular cartilage of the metacarpophalangeal joint.

Materials and methods

ANIMALS

Twelve female sheep (Merino $n=5$ and Merino−German Blackhead cross $n=7$), aged 4−7 years, weighing 67−95 kg, were used. Animals were free from disease and injury and were not fractious or otherwise unsuitable for this study (e.g., exhibited signs of lameness and/or pain on manipulation/palpation before induction of OA). They were group-housed in a semi-open barn located in Germany. They were fed a standard diet (hay or grass silage) and had water *ad libitum*. The sheep were divided equally into two groups of six animals each. The Institutional Animal Care and Use Committee (IACUC) approved the study.

THE GROOVE MODEL: ANAESTHESIA, SURGERY, AND POST-SURGICAL TREATMENT

In all sheep ($n=12$), OA was induced in both front metacarpophalangeal joints, i.e., lateral and medial, of the right limb. Sheep were anesthetized with a combination of xylazine and ketamin by intra-muscular route. Incisions in the skin (~4−5 cm) and joint capsule (~2 cm) were made, and care was taken to prevent bleeding and soft tissue damage as much as possible. The cartilage of the lateral and medial metacarpal was damaged with a Kirschner-wire (1.5 mm diameter) that was bent 90° at <0.5 mm from the tip. This procedure ensured that depth of the grooves was restricted to less than 0.5 mm. In utmost flexion, 10 longitudinal and diagonal grooves were made on the weight-bearing parts of the metacarpals[5–7]. There was no absolute visual control over the procedure, but macroscopic evaluation after necropsy of the animals showed similar patterns in all affected fetlock joints. After surgery, the synovium, fasciae, and skin were sutured. Following surgery, the fetlock was sprayed with CTC Blauspray and wrapped with an adhesive bandage. The contra-lateral unoperated joint served as an internal control.

The sheep were forced to load the experimental joint intermittently. Controlled exercise was performed on a concrete floor. For the first 3 weeks sheep were exercised on three legs, i.e., the left front leg was immobilized, for approximately 3 h for 3 days a week. During this period the animals became reluctant to walk (animals were lying down). Subsequently, the sheep were exercised on four legs, three times weekly for a total of approximately 2.5 h each week, in a training carrousel with an average speed of approximately 3.6 km/h, throughout the remainder of the study.

The severity of joint changes was evaluated at 15 ($n=4$) and 37 weeks ($n=6$) after surgery. Two animals had to be excluded from evaluation. One sheep of the short-term group (15 weeks) developed an (surgery related) inflammation of the right front fetlock on day 13 that did not respond to treatment and was euthanized 3 weeks after surgery. Results of a second sheep of the short-term group were omitted from the data analysis due to technical failures during harvesting of the cartilage and synovium (first animal in the procedure). Therefore, only four sheep could be used for evaluation in the short-term group and six sheep in the long-term group.

At the end of both follow-up periods, the sheep were humanely euthanized. Both front limbs were amputated and transported to the Netherlands. Synovium and cartilage were collected and processed within 24 h after amputation. Procedures were carried out under laminar flow conditions.

SYNOVIAL TISSUE ANALYSIS

Macroscopic synovial inflammation was evaluated using digital high-resolution photographs of synovium[9], by two observers unaware of the source of the photographs. Severity of inflammation was graded from 0 to 2 for colour, angiogenesis, and fibrillation: 0 = none, 1 = slightly, 2 = strong. The sum of these three individual scores averaged for the two observers (a maximum of six) was used as representative score of each joint and used for statistical analysis. Three synovial tissue samples per joint (fixed locations: medial, middle, and lateral) were fixed in 4% phosphate-buffered formalin (pH 7.0) and embedded in paraffin. Deparaffined sections were stained with haematoxylin−eosin. The histological sections were examined separately in random order and independently by two observers who were not aware of the source of the synovium. Each specimen was analyzed to determine the degree of inflammation, using the slightly modified[10] criteria described by Goldenberg and Cohen[11]. For assessing the overall grade, the three specimens from each fetlock joint were considered as a unit.

SYNOVIAL FLUID MMP ACTIVITY

Overall MMP enzyme activity was determined in synovial fluid samples using the fluorogenic substrate TNO211 as described previously[12].

CARTILAGE ANALYSIS

Macroscopic cartilage damage was evaluated using digital high-resolution photographs of the tibia and femur by two observers unaware of the source of the joints on the photographs[9]. Severity of cartilage damage of the femoral condyle was graded from 0 to 4: 0 = smooth surface, 1 = slightly fibrillated, 2 = fibrillated with shallow grooves, 3 = deep sharp grooves, 4 = deep sharp grooves with surrounding damage. Grading of cartilage damage of the tibial plateau was comparable: 0 = smooth surface, 1 = roughened, 2 = slightly fibrillated, 3 = fibrillated, 4 = damaged. Scores of the two observers were averaged (a maximum of four). This score was used as representative score of each photograph and was used for statistical analysis.

Cartilage samples for histological and biochemical analyses were obtained from pre-determined locations on the weight-bearing areas of the metacarpal and the phalanx of both experimental and control joints [Fig. 1(A)]. The locations were identically paired with the same location in the contra-lateral joint. Cartilage was cut as thickly as possible, while excluding the underlying bone. Samples were cut into full-thickness squares, kept in 200 μl culture medium (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 0.085 mM ascorbic acid, 2 mM glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin, and 10% heat-inactivated ovine serum), and weighed (3−10 mg; accuracy 0.1 mg).

For histology, four samples from the metacarpal and four from the phalanx from each fetlock joint were fixed in 4% phosphate-buffered formalin containing 2% sucrose (pH 7.0). Cartilage degeneration was evaluated in safranin-O−fast-green iron hematoxylin-stained sections by light microscopy according to the slightly modified[10,13] criteria of Mankin[14]. The tidemark between cartilage and bone was not present in the cartilage samples because bone was not included. Cartilage samples were not covered with pannus. Therefore, the maximum score that could be obtained was 11, instead of the original 14, when all criteria described by Mankin (including pannus, clefts to calcified zone, and tidemark crossed by blood vessels) could have been included. With respect to staining, paired control and experimental samples (of the same animal) were stained in the same safranin-O baths. Specimens were graded in random order by two observers unaware of the source of the cartilage samples. The average of the four specimens was used for statistical evaluation.

For biochemical analysis, the cartilage samples were cultured individually in 96-well culture plates (NUNCLON®, Denmark) in 200 μl culture medium. Cartilage explants were cultured according to standard procedures as described previously[15]. For metacarpal and phalanx, cartilage PG content, PG synthesis, retention of newly formed PGs, and PG release were determined and averaged for six explants per parameter[5].

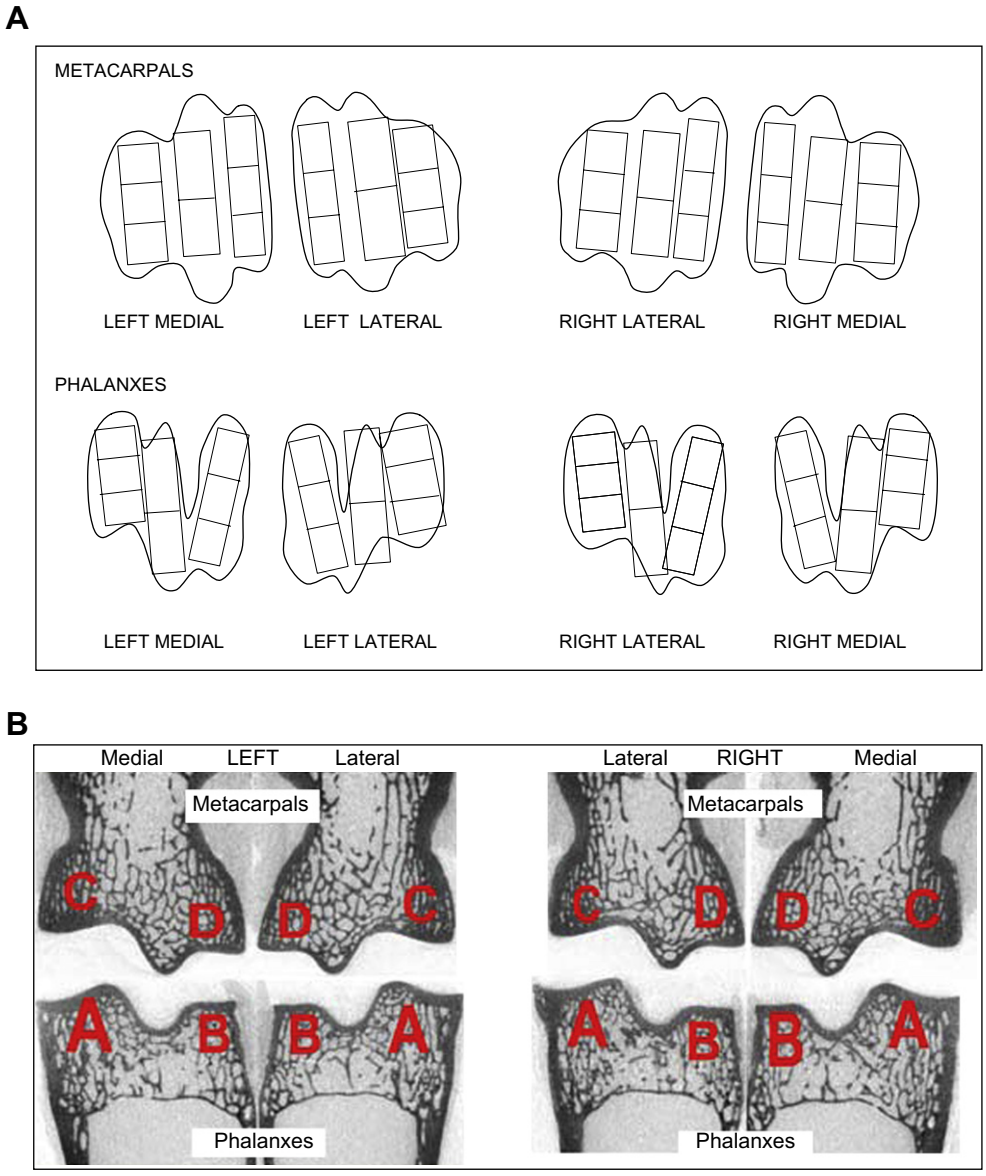

Fig. 1. A: Locations from which the pre-defined location cartilage were sampled from. B: Selected regions for micro-CT analyses that were analyzed for the metacarpal and phalanx.

PG synthesis rate

As a measure of PG synthesis rate, the rate of sulphate incorporation rate was determined *ex vivo*[15]. After 1 h of pre-culture, 148 kBq $Na_2^{35}SO_4$ (Perkin Elmer, Boston, MA, USA: NEX-041-H, carrier free) in 10 μl DMEM was added to each sample. After 4 h, the cartilage samples were washed with cold phosphate-buffered saline (PBS) and digested with papain for 2 h at 65°C. Glycosaminoglycans (GAGs) were precipitated by addition of cetylpyridium chloride (CPC), and $^{35}SO_4^{2-}$-labelled GAGs were measured by liquid scintillation analysis. The total sulphate incorporation rate of each cartilage sample was calculated using the specific activity of the medium and was normalized to time and wet weight of the explants. Synthesis rate is expressed as nmoles of sulphate incorporated per hour per gram wet weight of the cartilage (nmol/h g).

PG release

For determination of the release of the newly synthesized PGs as a measure for retention of these PGs, the release of $^{35}SO_4^{2-}$-labelled GAGs in the medium was determined. After labelling (see above), the cartilage samples were rinsed three times for 45 min in 1.5 ml culture medium and then incubated in 200 μl fresh culture medium without sulphate label for 3 days. Thereafter, the samples were washed with cold PBS, and GAGs were stained and precipitated from the medium with Alcian Blue, as described previously[15]. The $^{35}SO_4^{2-}$-labelled GAGs were measured by liquid scintillation analysis, and the release was normalized to the specific activity of the medium and the wet weight of the explants. The release of newly formed PGs was normalized to PG synthesis rate and expressed as percentage release of newly formed PGs in the 3 days (% new PG release).

For the total release of PGs, Alcian Blue staining of the medium as described above was quantified photometrically with chondroitin sulphate (Sigma C4384) as a reference. The total amount of GAGs released is expressed as a percentage of the original GAG tissue content (% GAG release).

PG content

As a measure of PG content of the cartilage samples the amount of tissue GAG was determined as described previously[15]. The GAGs in the papain digest of cartilage samples were stained and precipitated with Alcian Blue as described above. Blue staining was quantified photometrically by the change in absorbance at 620 nm. Chondroitin sulphate (Sigma C4383) was used as a reference. Values were normalized to the wet weight of the cartilage explants (mg/g).

Cartilage collagen damage

Degraded collagen was measured as described previously[16]. In short, after extraction of PGs, degraded collagen molecules in the insoluble collagen network were selectively digested with α-chymotrypsin at 37°C. The supernatant, containing the fragments derived from the digested collagen molecules, was removed and hydrolyzed, as was the insoluble matrix left after α-chymotrypsin digestion. The hydroxyproline content of both pools was used to calculate the percentage of degraded collagen present in the samples.

SUBCHONDRAL BONE CHARACTERISTICS

Phalanxes were separated from the metacarpal side of the joint, and the medial and lateral phalanxes were separated from each other with a scalpel. The metacarpal side of the joint was cut in half with a band saw resulting in a medial and a lateral part. Each metacarpal part and each phalanx were scanned at a resolution of 18 μm using the micro-CT device.

Using an automated computer procedure, the trabecular and cortical bones in the subchondral bone were separated for analyses. Elliptical cylinders containing trabecular bone and subchondral plate were selected using anatomical landmarks and analyzed. See Fig. 1(B) for the position of the cylinders. The sizes of the cylinders were as follows: cylinder A, 3.5 $\times$ 10.0 mm and approximately 6.3 mm high; cylinder B, 2.0 $\times$ 10.0 mm and approximately 5.1 mm high; cylinder C, 3.5 $\times$ 8.5 mm and approximately 3.7 mm high; cylinder D, 3.0 $\times$ 10.0 mm and approximately 2.7 mm high.

For the trabecular bone the following parameters were calculated:

- bone fraction (BV/TV): volume of trabecular bone (BV) divided by volume of trabecular bone and marrow cavity (TV) in the cylinder.
- trabecular thickness: mean thickness of trabeculae present in the cylinder, expressed in μm.
- structure model index (SMI): this is a measure of curvature of an object. The SMI value of an ideal plate is 0, and for an ideal cylindrical rod structure it is 3. A negative SMI value indicates a concave structure (e.g., a plate with holes in it).

For each animal, the average values of the four cylinders in the phalanx [A, B, B, A; Fig. 1(B)] were calculated for both control and experimental phalanxes. The same was done for the four cylinders in the metacarpal [C, D, D, C; Fig. 1(B)].

For the cortical bone in the elliptical cylinders (i.e., the subchondral plate), the thickness was calculated and expressed in μm. The presence or absence of osteophytes also was scored [Fig. 5(C)].

CALCULATIONS AND STATISTICS

Average absolute values for each of the parameters $\pm$S.E.M. (standard error mean) are given in the table. The paired Student's *t* test was used for all parameters except for macroscopy and histology to compare data of the experimental and contra-lateral control joints within each group. For macroscopy and histology the Wilcoxon signed rank test (for non-parametric data) was used. Mean delta or percentage change of experimental fetlock joints compared to control fetlock joint $\pm$S.E.M. of metacarpal and phalanx cartilage is presented in figures. The unpaired *t* test was used to analyze differences between the two groups of animals and between both follow-up times except for histology and macroscopy where the Mann–Whitney *U* test was used. *P* values less than 0.05 were considered statistically significant.

Results

CARTILAGE DAMAGE

Fifteen weeks after induction of experimental OA, the affected fetlock joints clearly showed macroscopic damage of the articular cartilage (fibrillation, localized partial thickness loss of cartilage tissue, and crumbling of the edges of the applied grooves) of the metacarpals in addition to the grooves that were surgically applied and still visible. No damage was found on the metacarpal cartilage of the control fetlock joints [representative photographs are depicted in Fig. 2(D vs A)]. Damage, although less pronounced, was also found on the phalanx of the experimental joints compared to the control joints [Fig. 2(E vs B)]. On average, the macroscopic cartilage damage was significantly more severe in the experimental joints compared to that in the control joints [Table I and Fig. 2(J), open bars]. At 37 weeks the macroscopic damage was comparable [Fig. 2(G and H)], neither scored different from the short-term group, nor for the metacarpals or phalanxes, when compared as a difference between experimental and control joints [Fig. 2(J)].

These macroscopic observations were confirmed by histological analysis. The average modified Mankin score of the cartilage degeneration in the experimental fetlock joints was mild but significantly higher compared to that of the contra-lateral control joints [Fig. 3(J), open bars]. The damage was clearly visible as depicted by a representative micrograph in Fig. 3(D and G vs A). Although not surgically damaged, mild cartilage degradation of the experimental phalanx cartilage was also found at both time points when compared to the contra-lateral control joints [representative micrographs are given in Fig. 3(E and H vs B)]. On average also for the phalanx histological cartilage damage was statistically significantly different from control joints [Table I and Fig. 3(J)]. No differences in histological cartilage damage between both time points were found [Fig. 3(J)].

SYNOVIAL INFLAMMATION AND SYNOVIAL FLUID MMP ACTIVITY

Macroscopically the synovial tissue showed mild signs of inflammation in the experimental joints compared to the contra-lateral control joints at 15 weeks, which diminished significantly at 37 weeks [Table I and Fig. 2(J)]. Figure 2(C, F, and I) depicts representative photographs for controls, the experimental 15 weeks and the 37 weeks follow-up groups. Over time inflammation diminished as reflected by a lower grade of inflammation in the long-term group compared to the short-term group ($P < 0.032$). This was confirmed by light microscopic examination of the synovial tissue [respectively, Fig. 3(C, F, I, and J); $P < 0.033$], where similar results were found. This reduced inflammation as detected by macroscopy and microscopy was further supported by the loss of synovial fluid MMP activity over time (Table I). At 37 weeks, a statistically significantly elevated MMP activity in the experimental joints compared to the contra-lateral control joints as found on 15 weeks, was not found anymore ($P < 0.02$).

PG TURNOVER AND COLLAGEN DAMAGE

Fifteen weeks post-surgery, synthesis of PGs of both metacarpals, as well as the phalanxes, was increased in the experimental joints compared to the contra-lateral control joints [Table I and Fig. 4(A)]. These changes were also present at 37 weeks although less pronounced; repair activity was statistically significantly impaired [Fig. 4(A)]. This increased PG synthesis, as observed for osteoarthritic cartilage, is ineffective as the release of these newly formed PGs was also enhanced in the experimental metacarpals and phalanx cartilage when compared to their controls (Table I). When the release of newly formed PGs was normalized to the synthesis rate (% newly formed PG release), a decreased retention of newly formed PGs in the experimental joints both at 15 weeks and at 37 weeks was observed. Although it was slightly less pronounced at 37 weeks this was not statistically significantly different from the change at 15 weeks [Fig. 4(B)]. Moreover, the release of the total amount of PGs was enhanced as a result of the experimentally induced joint damage in both metacarpal and phalanx plateau cartilage [Table I and Fig. 3(C)], again not statistically significantly different between 15 and 37

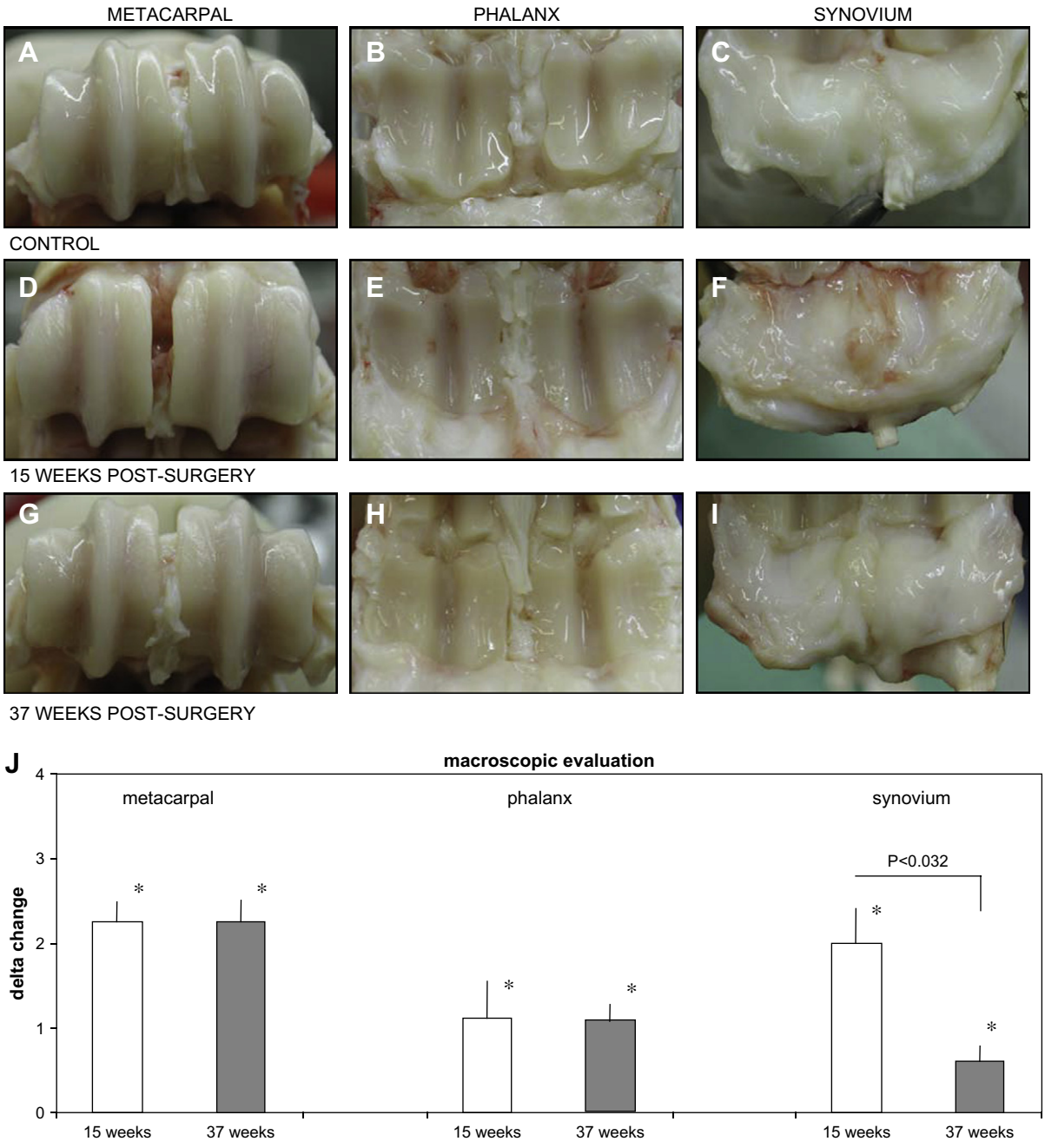

Fig. 2. Macroscopic changes as result of experimentally induced joint damage in the fetlock joint. Representative macroscopic views of the articular cartilage surfaces and synovium are shown. The metacarpal (A, D, and G), phalanx (B, E, and H), and synovium (C, F, and I) of the control (top), 15 weeks post-surgery (middle), and 37 weeks post-surgery (bottom) groups are presented. Average changes in macroscopic score of cartilage damage and synovial inflammation are depicted in Fig. 1(J). Open bars represent data at 15 weeks post-surgery and grey bars represent data at 37 weeks post-surgery. Mean $\pm$ S.E.M. values are presented. Asterisk indicates statistically significant changes compared to contra-lateral controls ($P < 0.05$). P values for differences between groups are given when statistically significant.

weeks post-surgery. The typical changes in chondrocyte activity as observed at 15 weeks post-surgery were not yet reflected in a decrease in the PG content of the cartilage tissue [Fig. 4(D) and Table I]. Collagen damage, on the other hand, was elevated in the experimental joints as compared to the control joints at 15 weeks, with statistically significant difference for the phalanx (Table I). After 37 weeks, the changes in chondrocyte activity were reflected in a statistically significant (25%) decrease in PG content [Table I and Fig. 3(D)], as well as an increase in collagen damage (Table I). Also for the surgically untouched phalanx the change in chondrocyte activity resulted in a statistically significant decrease in PG content, although less pronounced than in the metacarpal cartilage [Fig. 3(D)], and an increase in damaged collagen (Table I).

There was no statistically significant change in cartilage cellularity as measured by DNA content due to induction of joint damage at and between both time points. When results of the different parameters were expressed per DNA, similar results were obtained (data not shown).

Table I
Mean (±S.E.M.) absolute values of all parameters

	15 weeks post-surgery		$P<$	37 weeks post-surgery		$P<$
	Control	Experimental		Control	Experimental	
Macroscopic cartilage damage						
Metacarpal	0.0 ± 0.0	2.3 ± 0.3	0.029	0.2 ± 0.1	2.4 ± 0.2	0.013
Phalanx	0.0 ± 0.0	1.1 ± 0.4	0.050	0.1 ± 0.1	1.2 ± 0.2	0.023
Macroscopic synovial inflammation						
Joint	0.0 ± 0.0	2.0 ± 0.4	0.033	0.2 ± 0.1	0.8 ± 0.2	0.033
Histological cartilage damage (modified Mankin grade)						
Metacarpal	0.2 ± 0.1	3.9 ± 0.3	0.034	0.3 ± 0.3	3.8 ± 0.3	0.023
Phalanx	1.0 ± 0.3	2.6 ± 0.2	0.034	1.1 ± 0.4	2.4 ± 0.5	0.024
Histological synovial inflammation (Goldenberg and Cohen score)						
Joint	0.9 ± 0.0	2.6 ± 0.5	0.034	0.9 ± 0.3	1.4 ± 0.3	ns
Synovial fluid MMP activity [relative fluorescence unit (RFU)/s]						
Joint	0.09 ± 0.01	0.47 ± 0.11	0.020	0.18 ± 0.03	0.27 ± 0.04	ns
PG synthesis rate (nmol/h g)						
Metacarpal	4.9 ± 0.8	10.3 ± 1.4	0.022	3.1 ± 0.4	3.5 ± 0.4	ns
Phalanx	5.7 ± 0.8	12.6 ± 1.5	0.022	5.0 ± 0.3	6.7 ± 0.6	0.014
Newly formed PG release (%)						
Metacarpal	26.8 ± 1.8	33.3 ± 1.1	0.040	22.7 ± 2.3	26.0 ± 1.3	ns
Phalanx	28.3 ± 1.3	38.6 ± 2.7	0.040	26.3 ± 3.6	30.8 ± 2.0	ns
PG release (%)						
Metacarpal	15.6 ± 2.1	23.1 ± 1.9	0.022	13.9 ± 1.1	17.9 ± 0.6	0.014
Phalanx	17.2 ± 1.2	27.7 ± 2.8	0.022	16.6 ± 1.8	24.2 ± 1.2	0.014
PG content (mg/g)						
Metacarpal	19.9 ± 1.6	20.3 ± 1.3	ns	21.4 ± 1.5	17.1 ± 0.6	0.014
Phalanx	22.5 ± 2.1	22.7 ± 0.5	ns	22.7 ± 1.2	21.3 ± 0.7	0.037
Cartilage collagen damage (%)						
Metacarpal	7.85 ± 0.5	9.72 ± 0.67	ns	7.56 ± 0.43	10.31 ± 0.97	0.022
Phalanx	11.43 ± 1.56	16.97 ± 0.82	0.007	13.40 ± 1.59	17.76 ± 0.82	0.007
Bone fraction (BV/TV)						
Metacarpal	0.57 ± 0.03	0.54 ± 0.01	ns	0.67 ± 0.03	0.67 ± 0.02	ns
Phalanx	0.57 ± 0.02	0.56 ± 0.02	ns	0.56 ± 0.03	0.55 ± 0.02	ns
Trabecular thickness (μm)						
Metacarpal	364 ± 24	315 ± 23	0.001	360 ± 11	360 ± 9	ns
Phalanx	310 ± 24	315 ± 23	ns	301 ± 13	301 ± 9	ns
SMI						
Metacarpal	-2.0 ± 0.1	-1.6 ± 0.2	0.027	-0.9 ± 0.3	-1.0 ± 0.3	ns
Phalanx	-0.7 ± 0.1	-0.5 ± 0.2	ns	-0.4 ± 0.2	-0.5 ± 0.2	ns
Cortical bone thickness (μm)						
Metacarpal	867 ± 47	729 ± 59	0.036	595 ± 50	528 ± 47	ns
Phalanx	595 ± 50	528 ± 47	0.032	893 ± 40	735 ± 60	0.001

SUBCHONDRAL BONE

It appears that the subchondral bone is significantly changed as a result of the osteoarthritic process shortly (15 weeks) after surgery. All parameters point towards an impairment of bone quality, as shown by a decrease in metacarpal trabecular bone volume fraction (ns), trabecular thickness, and cortical bone thickness. At the same time, the SMI becomes less negative indicating that the inter-connected plates contain less closed cavities, which also indicates the loss of bone volume fraction and the reduction in trabecular thickness. These changes were most pronounced (statistically significant) at the metacarpal side, the side of the joint from which the cartilage was surgically damaged. Surprisingly, at 37 weeks, most of the subchondral bone changes present at 15 weeks had vanished; only cortical plate thickness was still decreased.

Marginal bone change, osteophyte formation, was found 15 weeks post-surgery in all but one experimental (osteoarthritic) metacarpal and in all phalanxes, while there were no osteophytes present in the left control metacarpals or phalanxes (data not shown). Osteophytes were mainly located on the outer sides of the phalanxes [near A in Fig. 1(B)] and the metacarpals [near C in Fig. 1(B)]. Some illustrative cross-sections are shown to clarify the osteophyte location [Fig. 5(C)]. At 37 weeks post-surgery, osteophytosis appeared progressive; osteophytes were present at both the lateral and medial sides of most of the metacarpals (five out of six). In one right metacarpal, only one osteophyte on the lateral side was present. In addition, in all right phalanxes, osteophytes were present on the lateral and medial sides. In one left control metacarpal, a very small osteophyte was present, and in the other five left metacarpals,

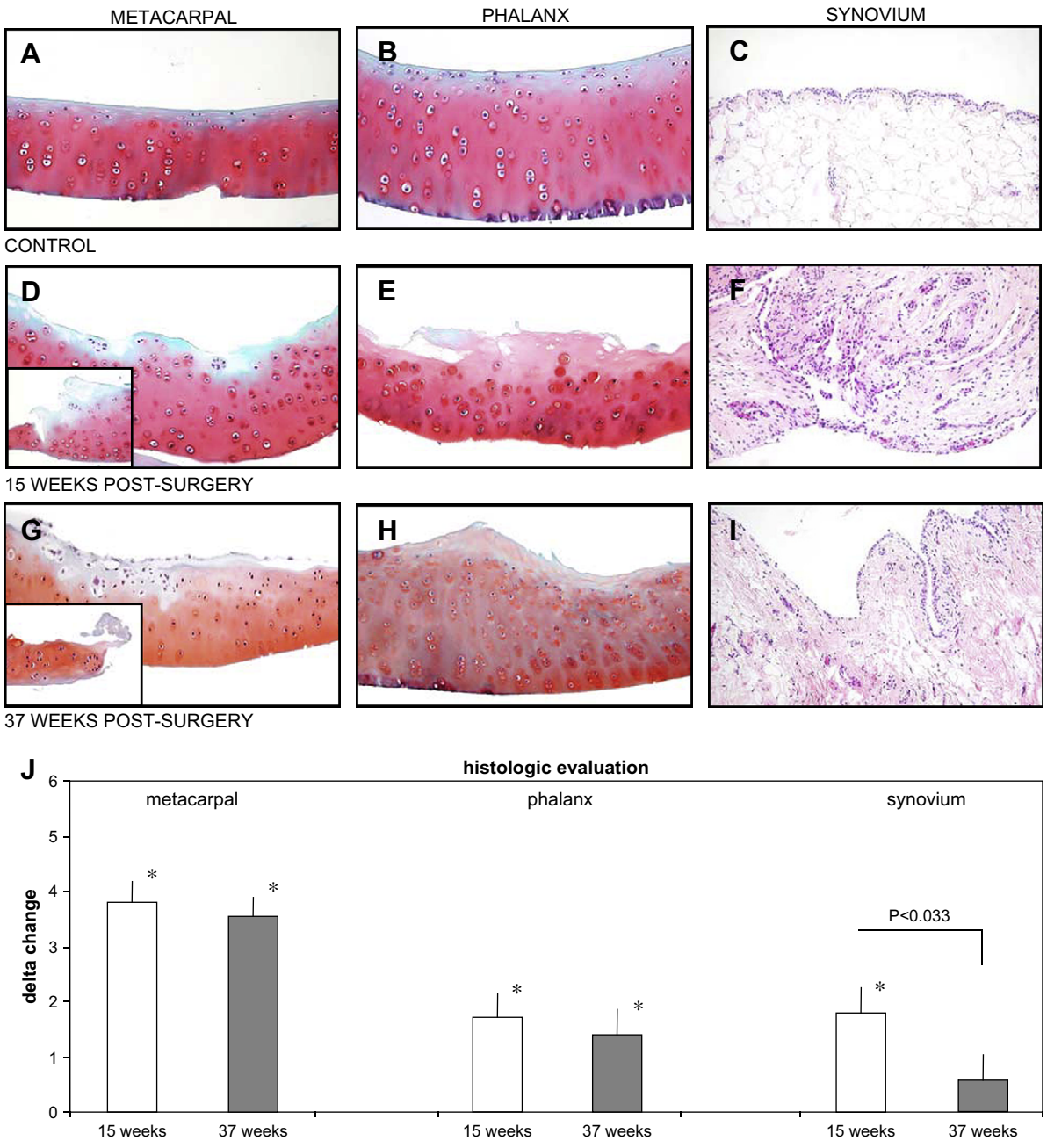

Fig. 3. Histological changes as result of experimentally induced joint damage in the fetlock joint. Representative light micrographs of the articular cartilage surfaces and synovium are shown. The metacarpal (A, D, and G), phalanx (B, E, and H), and synovium (C, F, and I) of the control (top), 15 weeks post-surgery (middle), and 37 weeks post-surgery (bottom) groups are shown. Average delta change of macroscopic score of cartilage damage and synovial inflammation are depicted in Fig. 1(J). Open bars represent data at 15 weeks post-surgery and grey bars represent data at 37 weeks post-surgery. Mean ± S.E.M. values are presented. Asterisk indicates statistically significant changes compared to contra-lateral controls ($P < 0.05$). P values for differences between groups are given when statistically significant.

no osteophytes were present. Furthermore, very small osteophytes were present in two left phalanxes, while in the other four left phalanxes no osteophytes were present (data not shown).

Discussion

This study demonstrates that experimentally (surgically) induced cartilage damage in the ovine metacarpophalangeal (fetlock) joint according to the 'Groove model'[5–7] results in clear macroscopic, histochemical, and biochemical features of joint degeneration. This study is the first time that the Groove model has been applied to a metacarpophalangeal joint. OA in the metacarpophalangeal joint is more frequently seen at older ages and after intense exercise[17] and is a major economic problem in veterinary medicine for horses[4]. Despite the development of experimental joint damage with features of OA, application of the Groove model in the ovine metacarpophalangeal joint has its limitations.

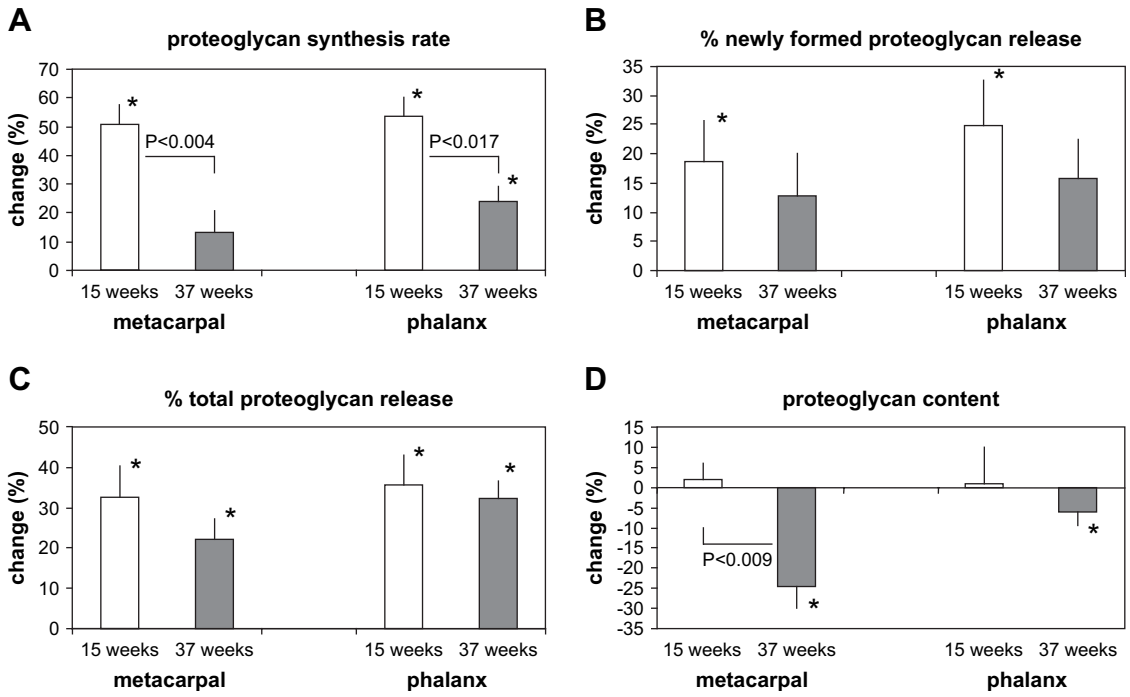

Fig. 4. Biochemical changes of experimentally induced joint damage in the fetlock joint. Percentage change of PG synthesis rate (A), percentage release of newly formed PGs (B), percentage total release of PGs (C), and PG content (D) of metacarpal and phalanx at 15 weeks post-surgery (open bars) and 37 weeks post-surgery (grey bars) are depicted. Mean ± S.E.M. values are presented. Asterisk indicates statistically significant changes compared to contra-lateral controls ($P < 0.05$). *P* values for differences between both time points are given when statistically significant.

Cartilage on the ovine metacarpophalangeal joint surfaces is much thinner than the cartilage surfaces of the canine knee joint for which the Groove model was originally developed. To keep the 'grooves' restricted to the cartilage without damaging the subchondral bone is more difficult in this very thin metacarpal cartilage. Restricting the surgically applied damage to the cartilage has been shown to be essential in the development of joint damage in this model[7,18] because bone damage might result in the release of precursor cells that could facilitate cartilage repair[19,20]. In fact the possibility that occasional subchondral bone damage may have influenced the development of joint damage cannot be entirely ruled out. The thin cartilage also resulted in other technical difficulties: harvesting the cartilage was complicated, which caused the loss of the data from one (first analyzed) animal in the 15 weeks group. In this respect the 'fetlock Groove model' might best be applied in larger animals like horses instead of sheep, because they have thicker cartilage[21]. Notwithstanding these difficulties, degenerative joint damage was clearly present in this model.

The results demonstrated a relatively slow progression of joint damage. Although cellular changes were already present at 15 weeks, an actual decrease in PG content in both joint compartments and collagen damage at the metacarpal side was only detected after 37 weeks. This observation did not corroborate the macroscopic and microscopic findings which showed an equal degree of joint degeneration at 15 and 37 weeks post-surgery. The fact that these parameters are less distinctive in determining a certain degree of degeneration might account for this apparent discrepancy. In the dog knee joint, a similar grade of joint damage, including biochemically determined PG loss and collagen damage demonstrating impaired cartilage tissue integrity, was observed as soon as 10 weeks post-surgery[5]. However, it should be clear that although the progression of degenerative features is suggestive it is not proven that these features eventually lead to full-blown OA and may even be the expression of non-progressive cartilage damage.

It appears on average that the changes in the surgically untouched phalanxes were slightly less pronounced than the changes in the surgically grooved metacarpals. This result corroborates the development of cartilage degeneration in the canine Groove model. Damage of the phalanx was most likely the result of incongruity (a mechanical component) of the articular surfaces after condyles had been damaged. MMP, which are able to damage collagen and PGs[22,23], might be involved as well (biochemical component), as demonstrated by elevated MMP activity in the synovial fluid (Table I), especially in the early stages.

Very interesting was the observation that synovial inflammation clearly decreased over time. This result is consistent with the canine Groove model. This decrease can be advantageous when treatment strategies are aimed at chondroprotection and cartilage repair instead of directly at restraining inflammation. In models in which damage is for a significant part dependent on the synovial inflammation[24], evaluation of cartilage-directed treatment strategies might be hampered by the prominent role the inflammatory activity has.

Osteophyte formation is an important issue in radiographic characterization of OA[25], appeared clearly progressive over time. As demonstrated in the canine version of the Groove model, osteophytes are not the results of the surgery, as in sham operated animals osteophytes did not develop (manuscript in preparation) suggesting that they are an integral feature of development of OA.

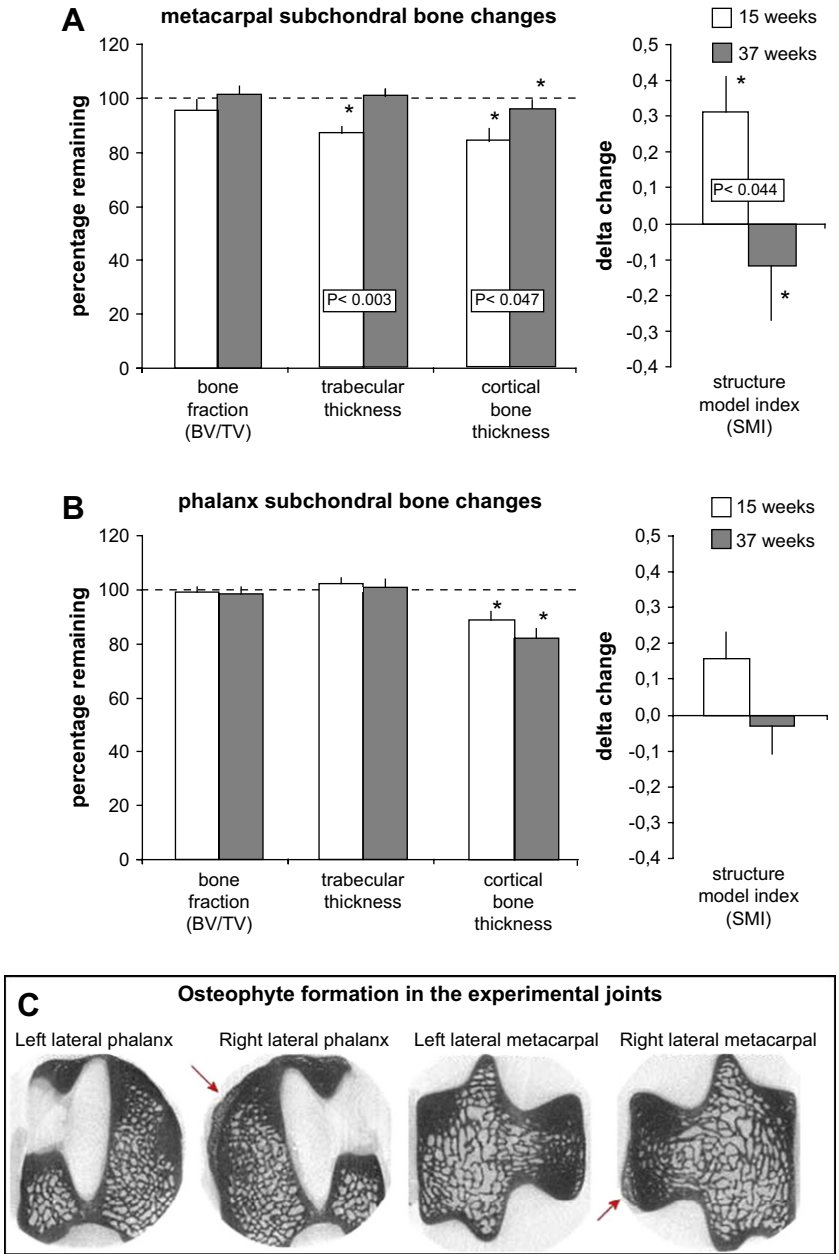

Fig. 5. Subchondral bone changes of experimentally induced in the joint damage fetlock joint. Change of bone parameters of the metacarpal (A) and phalanx (B) at 15 weeks post-surgery (open bars) and 37 weeks post-surgery (grey bars) are depicted. Mean ± S.E.M. values are presented. Asterisk indicates statistically significant changes compared to contra-lateral controls ($P < 0.05$). C: Representative micro-CT cross-section of the lateral phalanx and metacarpal of the experimental (right) and control (left) joints illustrating the localization of osteophytes as indicated by the arrows.

The subchondral bone changes as determined by micro-CT at 15 weeks post-surgery pointed to impaired bone quality. A decrease in trabecular bone fraction, trabecular thickness, and cortical bone thickness demonstrate this clearly. The SMI becomes less negative indicating that the inter-connective plates contain fewer closed cavities, reflecting the loss of bone volume fraction and the reduction in trabecular thickness. All these changes were most pronounced in the surgically damaged metacarpal and were hardly evident in the phalanx.

Thirty-seven weeks after surgery the trabecular bone changes were more or less normalized. From this result, it might be concluded that the early 15-week changes are (at least in part) dependent on possible damage to the bone during surgical procedures. Because histology did not include subchondral bone this could not be verified by the selection of histological sections taken. On the other hand the results may also represent early changes that are compensated for during the development of OA. The latter hypothesis would fit with the findings that the cortical bone thickness is decreased at 15 weeks and further decreased at 37 weeks at the phalanx (surgically untouched) side. In that respect, cortical plate changes may behave differently from subchondral trabecular bone changes, as was

also recently observed for the canine Groove and ACLT model of OA (manuscript submitted for publication). More detailed studies of the exact changes in subchondral bone and their role in the process of OA are needed. In this respect it should be kept in mind that the progression of OA may follow different pathways with a different series of events, also in bone architecture. It is also of major importance to relate changes in bone architecture to changes in loading of the affected joints, as unloading, due to the surgical procedures or the actual process of OA (pain), may have a major impact on bone architecture. The other way around changes in bone architecture may affect pain and with that loading.

In conclusion, based on this first (specifically technical) experience, the metacarpophalangeal Groove model is not recommended for use in sheep. However, the results demonstrate that the Groove model can be applied to the metacarpophalangeal joint as features of joint damage were very similar to those found in the canine knee Groove model. Whether these features are indeed the herald of progressive OA or just the expression on non-progressive cartilage damage remains to be established. Irrespectively, the use of larger animals, such as horses, may significantly facilitate the development of technical procedures and the reliability of the model.

Conflict of interest

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