The effect of body mass and physical activity on the development of guinea pig osteoarthrosis

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Submitted 02-04-22. Accepted 02-12-03

ABSTRACT **We quantitatively evaluated the morphological and biochemical effects of body mass and physical activity on spontaneously developing guinea pig osteoarthrosis (OA). 6-month-old male guinea pigs were allocated to 3 groups: controls (C) living under standard laboratory conditions with food ad libitum; mobilized animals (M) allowed unrestricted motion in large rooms with food ad libitum; and a diet group (D) weight-matched with the M-group. At 9- and 12-months of age they were killed and the left proximal tibia was processed for quantitative histology and the right tibial articular cartilage for analyses of glycosaminoglycan (GAG).**

OA mostly occurred on the medial condyle's central part not covered by the meniscus. The thinnest cartilage was found in 12-month-old M-animals (M12), which had 60% of the central cartilage surface affected by lesions that extended down to the mineralized cartilage. C12 had 25% exposed mineralized cartilage and D12, 2%. Subchondral bone density followed the loading patterns—the highest in M12 and lowest in D12. M12 had the lowest cartilage GAG concentrations.

Load appears to be a key external factor in guinea pig OA. An increase in physical activity may be chondroprotective in the early phase, but harmful when fibrillations eventually have developed. This is underscored by the extensive OA changes in M12, although these animals weighed about the same as D12 (which had the least extensive OA). Therefore, a reduction in body mass seems to retard the progression of OA in animals, which are mainly subjected to a static load (C12 and D12), but not sufficiently in animals with a more dynamic load (M12). Changes in morphological patterns are paralleled by changes in GAG concentration, which probably reflect the metabolic capacity of the cartilage.

Osteoarthrosis (OA)—a nonspecific term for late joint destruction—is one of the commonest causes of pain and disability. Age, load, trauma and heredity have been correlated with human OA (Felson 1998). However, since OA develops during several decades, confounding factors hamper epidemiological studies. For ethical reasons, it is not possible to take biopsies from loaded human joint cartilage. Consequently, several animal models have been used, some involving graded injuries to the joint (Brandt et al. 1991), and others changing load patterns (Radin et al. 1984). There are also many animal models which spontaneously develop OA—e.g., Hartley guinea pigs that develop severe medial tibial condyle arthropathy resembling human OA at 12 months (Bendele and Hulman 1988, de Bri et al. 1995). A change in load seems to alter the natural history of guinea pig OA. Diet restriction (Bendele and Hulman 1991), femur valgus osteotomy (transferring the load from the medial to the lateral side), and below knee amputation (Wei et al. 1998b) all retard the development of OA. Exercise also seems to affect the outcome (Hyttinen et al. 2001).

Biochemical changes affecting cartilage integrity are likely to precede morphological ones. The proteoglycan (PG) aggrecan is the major non-collagenous component in cartilage. It consists of a central protein core, to which are attached highly negatively-charged glycosaminoglycan (GAG) chains, mainly chondroitin sulfate. Cartilage compressive stiffness depends on GAG content (Kempson et al. 1970). The GAG concentration declines in advancing human (Venn and Maroudas 1977) and guinea pig OA (Wei et al. 1997).

Experimental groups

Groups	Abbreviation	n	Body weight (kg) [a]	Design
Control, 9 months	C9	8	1.3 (0.11)	Single-caged (33 × 55 cm), standard laboratory conditions, food ad libitum
Control, 12 months	C12	6	1.4 (0.08)	
Mobilized, 9 months	M9	5	0.89 (0.05)	At 6 months shared cage (240 × 160 cm), which allowed free mobility, food ad libitum
Mobilized, 12 months	M12	10	0.85 (0.05)	
Diet, 9 months	D9	8	0.88 (0.05)	Single-caged (33 × 55 cm). Regulated feeding from 2 months of age aiming at the same weights as M9 and M12
Diet, 12 months	D12	8	0.84 (0.05)	

[a] Mean (SD)

We quantitatively evaluated the morphological and biochemical effects of body mass and physical activity on the development of guinea pig OA.

Animals and methods

We studied 6 groups of male Hartley guinea pigs (Sahlins, Malmö, Sweden) (Table): controls (C9 and C12) and diet groups (D9 and D12) fed under standard laboratory conditions, and freely mobilized animals (M9 and M12) reared in a large room. All animals had standard laboratory food, C and M ad libitum. D were weighed weekly from 2 months of age and the food supply was adjusted to ensure the same final weights as M9 and M12 (Table).

The animals were killed by an intraperitoneal injection of pentobarbital at 9 or 12 months. Following dissection, the right tibial plateau was immediately snap-frozen in liquid nitrogen for biochemical analyses, while the left one was fixed in neutral buffered 4% paraformaldehyde and processed for light microscopy. The experiments were approved by the Board of Ethics for Animal Experiments in Stockholm-South.

The left proximal tibiae were cut sagitally in halves (the medial and lateral condyles). Each condyle was serially sectioned at intervals of 250 μm (section thickness 5 μm) with random start from the intercondylar area. The sections were stained with hematoxylin and eosin, coded, mixed and analyzed blindly, using a light microscope (×150) with a computerized image analyzer (CAST-Grid, Olympus, Aarhus, Denmark). The middle third of the first 5 sections, without cruciate ligament remnants, was defined as the central portion of the condyle, corresponding to an area of 3–4 mm^2. All measurements were made in this central portion. The proportion of intact cartilage and exposed mineralized cartilage was measured with a digitalized ruler. The volume density of subchondral bone, including the subchondral bone plate and subchondral trabecular bone down to the physis, was measured by point counting (Gundersen et al. 1988). The average cartilage thickness was estimated by dividing the cartilage volume by the projection of the corresponding surface area. One C9 and one M12 condyle could not be processed because of technical problems.

Cartilage from the central portion of the medial tibial condyles was punched out with an instrument for taking biopsies (diameter 2 mm). Care was taken not to include subchondral bone. The tissue was solubilized by papain digestion at 65 °C for 3 h; the digestion buffer contained 0.05 M phosphate at pH 7.0, 0.05 M EDTA and 0.005 M cystein. After inactivation of the remaining enzyme at 100 °C for 5 min, one aliquot was kept to analyze hydroxyproline (collagen) (Stegeman and Stadler 1967), and the glycosaminoglycans (GAG) were precipitated from the remaining digest, by adding 4 volumes of ethanol. The precipitates were then digested overnight at 37 °C, using a mixture of chondroitinase AC and ABC (0.1 U/mL of each enzyme) in 0.3% Tris buffer at pH 7.5. High-performance liquid chromatography was used to characterize the sulfation pattern and uronic acid content (GAG). One C12, D9, D12 and two M12 condyles were excluded because of technical problems when processing the tissue.

The variables GAG concentration, cartilage thickness and bone density were analyzed, using a two-way factorial ANOVA. The factors were

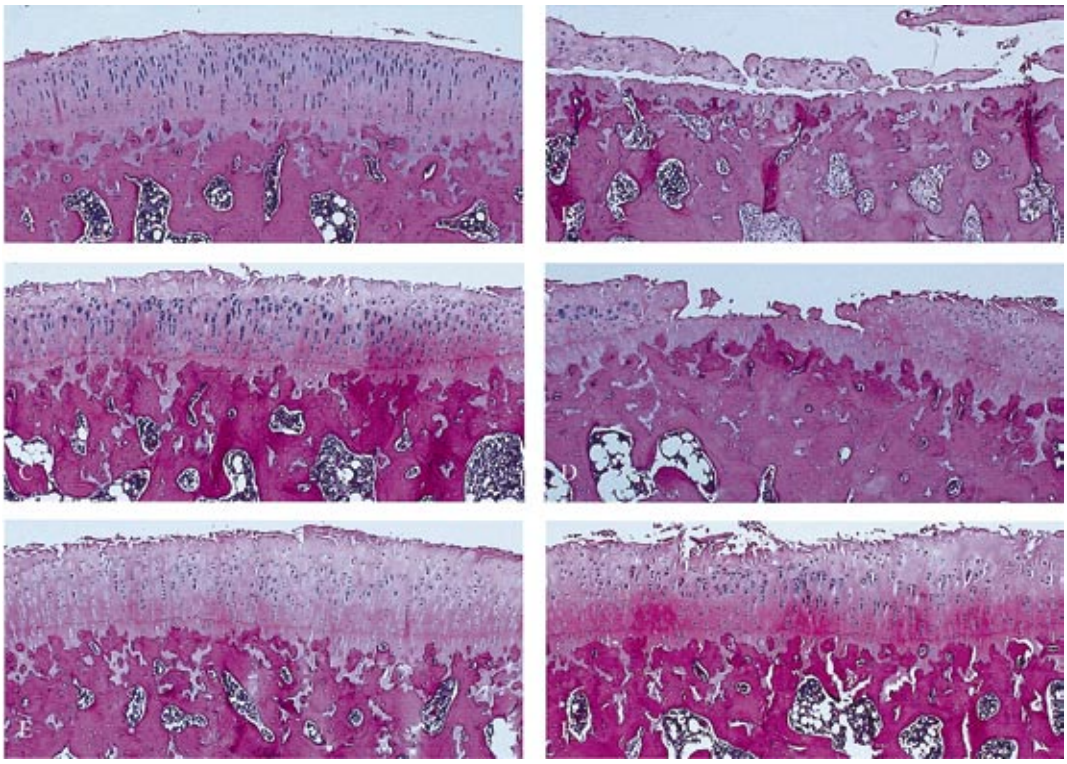

Figure 1. Guinea pig articular cartilage from the central third of the medial tibial condyle in the 9- (left column) and 12-month-old (right column) mobilized (A, B), control (C, D) and diet animals (E, F).

Group with the levels M, C and D and Months with the levels "9 months" and "12 months". In the event of significant interaction, simple effects were studied—i.e., the effects of one factor holding the other factor fixed. The p-values were then corrected by the Bonferroni procedure. When the F-ratio for the factor group was significant, the post hoc Tukey test was done to make all pair-wise comparisons among means.

The variances in the treatment groups were not homogeneous for the variables intact cartilage and exposed mineralized cartilage. We therefore used non-parametric statistics for these variables. To test the effect of groups in the various age groups, we used Kruskal Wallis ANOVA by ranks, followed by multiple comparisons of the groups. To test the effect of age in the groups, the Mann-Whitney U-test was done. The p-values were adjusted by the Bonferroni procedure.

Results

The animals did not seem to be severely disabled as regards their knee joints—i.e., they could move without difficulty. The mobilized animals were more active, they ran and jumped, than the sedentary ones in cages.

The morphological findings in the cartilage varied between the groups (Figure 1). The proportion of intact cartilage medially declined with age in M ($p = 0.008$), and no change was seen in C and D, but the variation was wide in each group. D9 had less intact cartilage than M9 ($p = 0.03$) (Figure 2A). No group had lateral lesions at 9 months, but at 12 months, both C12 and M12 had changes in cartilage surfaces (Figure 2B). The thickness of the medial condyle cartilage declined between 9 and 12 months in M ($p = 0.02$) and was thinner in M12 than in C12 and D12 ($p < 0.001$) (Figure 3A). The lesions that exposed the mineralized cartilage were mainly found medially and the area of the lesions increased between 9 and 12 months in M ($p =$

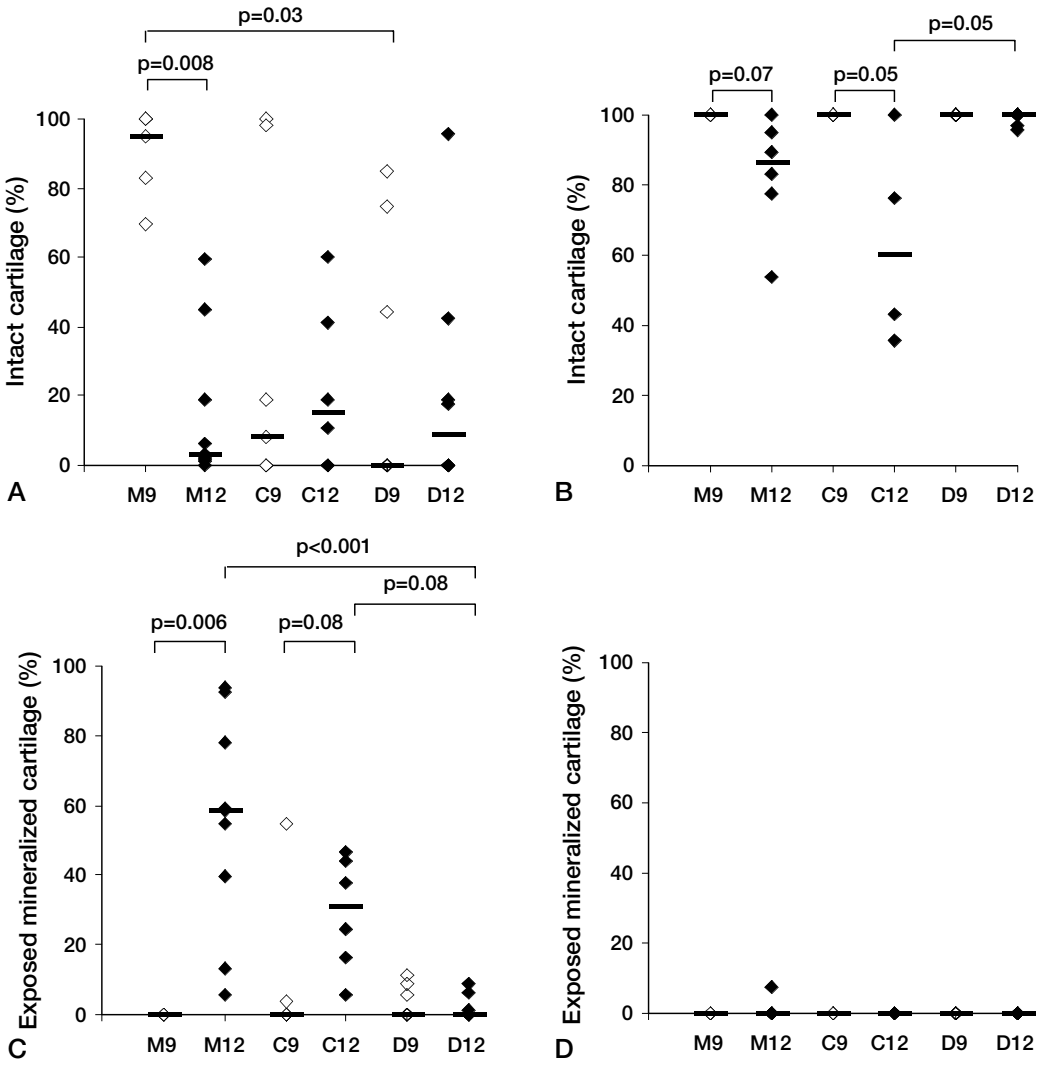

Figure 2. Proportion of intact and exposed mineralized cartilage in the central third of the medial (A, C) and lateral tibial condyles (B, D) in the 9- and 12-month-old animals. The short line denotes the median value in each group. M = mobilized, D = diet and C = control animals. Statistically significant differences between the groups are indicated when p-values are less than 0.1.

0.006) and C ($p = 0.08$). The area of the lesions were about the same in M12 and C12, but both had larger lesions than D12 ($p < 0.001$ and $p = 0.08$) (Figure 2C). The density of the subchondral bone increased medially between 9 and 12 months in M ($p = 0.004$), decreased in D ($p = 0.04$) and remained unchanged in C. M12 had greater bone density than C12 ($p = 0.02$) and D12 ($p < 0.001$), and D12 had less than C12 ($p = 0.01$) (Figure 3C).

The GAG concentration (uronic acid/hydroxyproline) in the medial tibial condyle varied considerably between the animals in a similar manner to the morphometrical patterns. It declined between 9 and 12 months in M ($p = 0.02$), but remained unchanged in C and D. M12 had lower GAG concentration than D12 ($p = 0.003$) (Figure 4A). The ratio of 6–4 sulfated chondroitin sulfate was higher in C9 than in D9. The ratios declined in C between 9 and 12 months (Figure 4B).

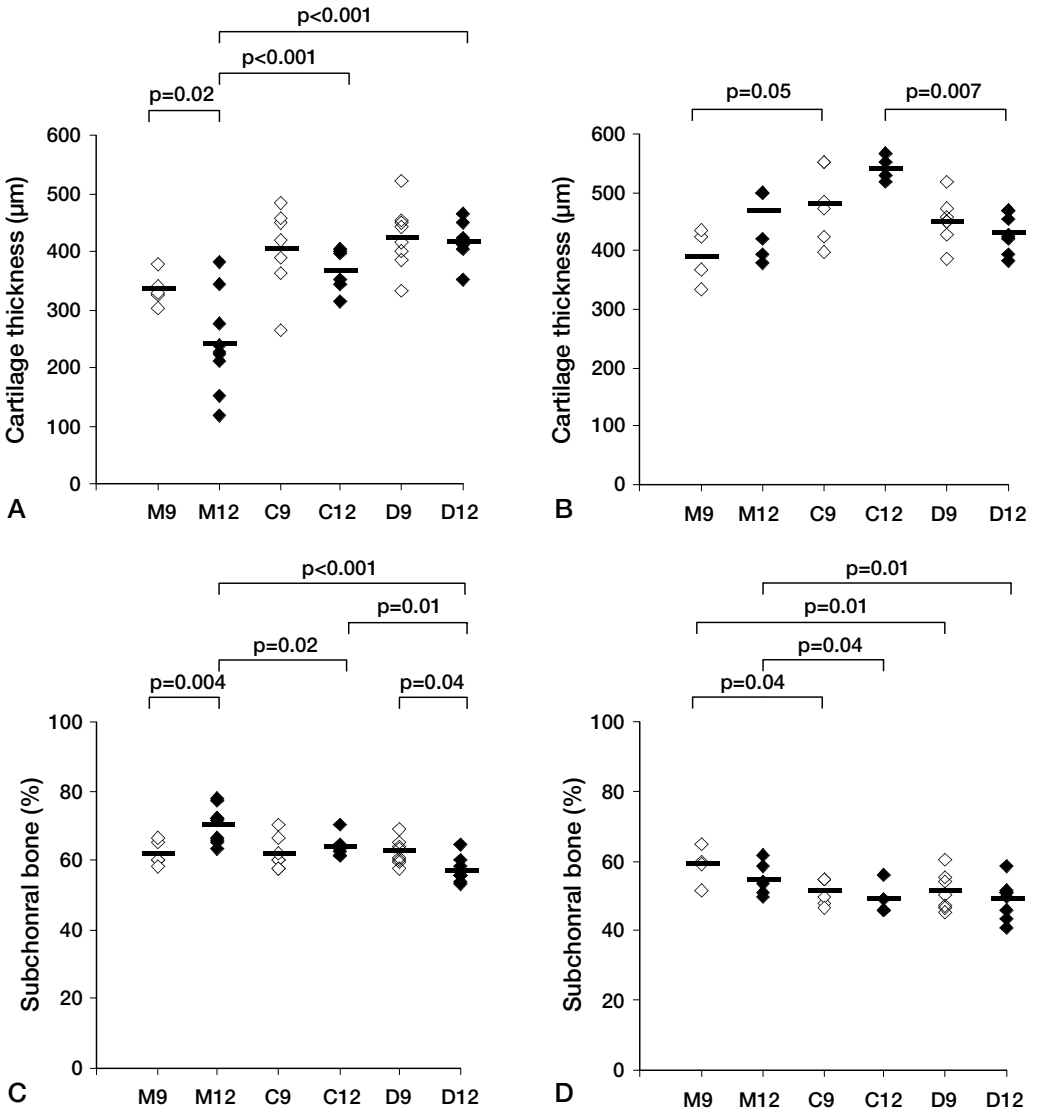

Figure 3. Cartilage thickness and subchondral bone density in the central third of the medial (A, C) and lateral tibial condyles (B, D) in 9- and 12 month-old animals. The short line denotes the average value in each group. M = mobilized, D = diet and C = control animals. Statistically significant differences between the groups are indicated when p-values are less than 0.1.

Discussion

By 12 months, all animals had developed OA in the medial condyle. Cartilage thickness, as a measure of cartilage destruction, was found to be thinnest in M12 (65% of that in C12). However, thickness per se may not accurately mirror early changes, because cartilage swells in early OA (Venn and Maroudas 1977). This was evident in C9 and D9, which had less intact, but thicker cartilage than M9. Therefore the OA process seems to have accelerated between 9 and 12 months in M, but remained about the same in D.

The area of exposed mineralized cartilage—a measure of more advanced OA—showed a similar pattern with no lesions in M9, minor lesions in C9, D9 and D12, and more extensive lesions in M12 and C12. Consequently, guinea pig OA appears to be worsened by physical activity (frequent joint loading) (M), and large body mass (constant

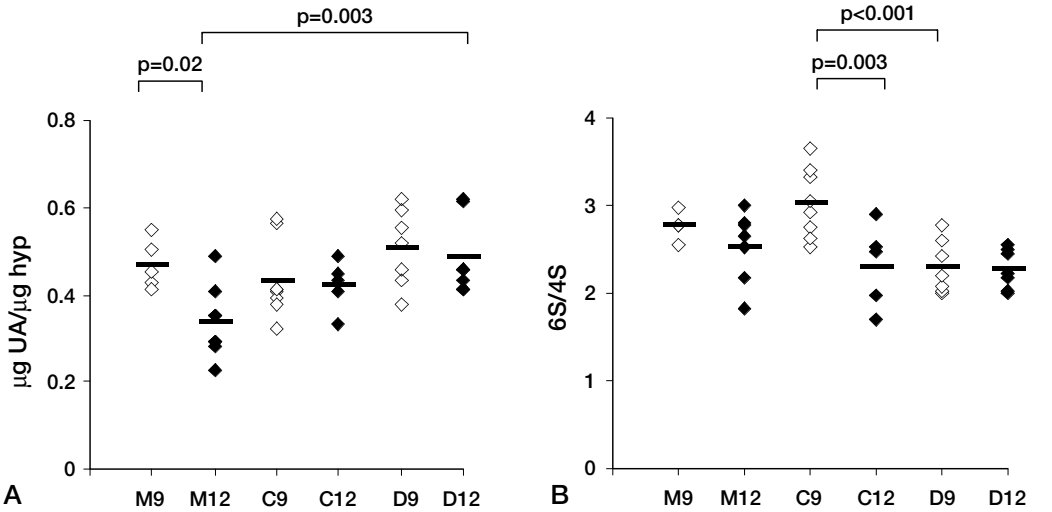

Figure 4. Proteoglycan content, µg uronic acid per µg hydroxyproline (hyp), and the ratio of 6 and 4 sulfated chondroitin sulfate (6S/4S) in the central third of the medial tibial condyle in 9- and 12-month-old animals. The short line denotes the average value in each group. M = mobilized, D = diet and C = control animals. Statistically significant differences between the groups are indicated when p-values are less than 0.1.

static joint loading) (C). Although it is impossible to quantify load and isolate the cumulative effects during several months it seems likely that C and D had a more static loading, despite their freedom to move about in their cages. The leaner M had a different pattern of motion with rapid accelerations and decelerations.

Exercise may be chondro-protective in the early phase, as seen in M9 and in hamsters developing spontaneous OA (Otterness et al. 1998). The finding of higher collagen fibril assembly in the superficial cartilage of physically active young guinea pigs supports this view (Hyttininen et al. 2001). However, when fibrillation develops, frequent loaded joint motion may become harmful, as shown by the extensive OA lesions seen in M12. This is especially interesting, since the body weight of M12 was similar to that of D12, which had the least severe OA lesions at this age. Our results reinforce previous qualitative observations of delayed development of OA in diet-restricted guinea pigs (Bendele and Hulman 1991) and that surgically-induced load changes can modify the natural history (Wei et al. 1998b). Our findings also accord with epidemiological studies showing an association between overweight as well as vigorous physical activity and knee OA (Felson et al. 1997, McAlindon et al. 1999).

The difference between groups in loading of the medial condyle is evident from the subchondral bone density results—i.e., the higher the load, the greater the bone density. On the other hand, nutritional effects of food restriction cannot be entirely excluded as a cause of bone changes, although this seems to be unlikely, since the laboratory fodder is protein-rich and fortified with calcium and vitamin D.

An increase in subchondral bone density has been suggested as a pathogenetic mechanism in OA (Radin et al. 1984). We found no such changes in our study. In particular, the medial subchondral bone densities were similar in the groups at 9 months. Moreover, below-knee amputation does not stop the progression of OA, but cause a reduction in subchondral bone thickness (Wei et al. 1998b). Thus OA and changes in subchondral bone density may be parallel phenomena in this model.

The GAG concentration, expressed as uronic acid related to hydroxyproline, decreased from 9 to 12 months in the M animals in accordance with previous results on guinea pig OA (Wei et al. 1997) and findings in human OA (Mankin et al. 1971). This reduction had probably occurred at 9 months in C, while the levels remained unchanged in D, which may have reflected the increase in PG synthesis seen in early guinea pig OA (Wei et al.

1998a). Although guinea pig OA shows structural changes like those in its human counterpart, there are conspicuous differences in the development of the disease—i.e., its high incidence and low variability in terms of temporal pattern and anatomic distribution. However, we think that it is a useful model for more studies on the pathomechanisms of OA, especially in younger animals, when, in principle, the changes can still be treated. Chondroprotective agents would have a great clinical effect since current drugs affect only pain and inflammation.

This study was supported by Anna-Greta Crafoord's Foundation for Rheumatologic Research, Gun and Bertil Stohne's Foundation, King Gustaf V:s 80 years Anniversary Foundation, Swedish Research Council (proj. no. 12231), Swedish Rheumatic Association, Filip Lundberg's Foundation, the Kempe Foundations, and the Swedish National Center for Research in Sports.

No competing interests declared.

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