Concentrations of proteoglycan fragments in relation to maturation, sex and time of day

Physiologic variations in knee joint fluid of rabbits

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We analyzed the concentrations of proteoglycan fragments in knee joint fluid in 142 rabbits to investigate the effect of physiologic variations—i.e., maturation, sex and time of day. The concentrations of proteoglycan fragments differed significantly between young, adolescent and adult animals and showed an inverse correlation to the stage of maturation of the rabbit. Adolescent male rabbits had higher concentrations than age-matched females. Morning and evening samples had similar concentrations. No relation was found between the proteoglycan fragment concentrations in joint fluid and the cartilage mass. The proteoglycan fragment concentrations in knee joint fluid apparently reflect the metabolic status of growing articular cartilage. There are considerable physiologic variations associated with maturation and sex, and these need to be taken into account when using the proteoglycan fragment concentration as a marker for joint diseases.

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The concentrations of proteoglycan fragments (PG) in joint fluid have been used as a marker for early diagnosis of knee arthrosis. Although arthrosis was usually associated with high PG concentrations (Lohmander et al. 1989, Dahlberg et al. 1992), staging of this disease was not successful with this marker, except in a rabbit experiment, where higher concentrations were found in more advanced than in early stages of arthrosis (Messner et al. 1993). Many factors have been found to influence the PG concentrations in joint fluid, such as time elapsed from initial injury to sampling, operative trauma and synovial clearance rate (Lohmander et al. 1989, Odenbring et al. 1991, Myers et al. 1995). Furthermore, there is no knowledge about normal physiologic variation of this marker because of the lack of controls. However, such data are mandatory if PG concentrations are to be used as a joint fluid marker. The rabbit knee is one of the commonest models for the pathogenesis of arthrosis. Thus, we investigated the influence of maturation, sex and time of day on the PG concentrations in the knee joint fluid of healthy rabbits.

Animals and methods

142 normal New Zealand white rabbits were used. Maturation-dependent differences were analyzed in equal numbers (3 × 21) of young, adolescent, and adult animals of both sexes (Table 1). 12 male and 12 female rabbits (aged 19 *1.0* weeks, mean SD) were used to test possible differences between sexes. Another 14 rabbits were used (aged 20 *0.4* weeks) to evaluate differences between mornings and evenings. In 7 of the latter animals, joint fluid was sampled first in the evening and again 2 weeks later in the morning. In the other 7, the order of sampling was the reverse. All rabbits were healthy, moved freely in separate cages and received a standard diet. The experiment was approved of the local ethics committee.

For the joint fluid sampling, the rabbits were anesthetized by an intravenous injection of ketamine and xylazine chloride (15 mg/kg and 1.5 mg/kg, respectively) and then weighed. After shaving of both knee joints, 1 mL of sterile 0.9% NaCl solution was injected into each knee from the medial side of the patellar tendon, and the knee was subjected to a 20 times full range of motion. The joint fluid was then aspirated. Keeping the needle in place, the above procedure was repeated. The two samples from each knee were combined and stored at –20 °C until analyzed. The PG concentrations in joint fluid were analyzed by precipitation of sulfated glycosaminoglycan containing proteoglycan fragments with Alcian blue (AB-method; Björsson 1993). Chondroitin sulfate (Sigma C4384) was used as standard.

Table 1. Age, weight, joint fluid volume, and PG concentrations in the different age groups, mean *SD*

	n	Age (weeks)	Weight (kg)	Volume (mL)	PG (μg/mL)
Young	21	140.8[a]	2.4 0.5[a]	1.3 0.3	5.2 1.2[a]
Adolescent	21	212.7[b]	3.4 0.4	1.3 0.1	4.1 0.9[b]
Adult	21	341.1	3.6 0.3	1.3 0.2	3.1 0.7

[a] P < 0.001 compared to adolescent and adult animals.
[b] P < 0.001 compared to adult animals.

Table 2. Sex, time of day and PG concentrations, mean *SD*

	n sample	Joint fluid volume (mL)	PG (μg/mL)
Male	12	1.3 0.2	5.2 0.9[a]
Female	12	1.3 0.2	4.0 0.7
Morning	14[b]	1.3 0.1	4.2 0.9
Evening	14[b]	1.3 0.2	3.8 1.0

[a] P < 0.01 compared to females.
[b] The same 14 rabbits were used for joint fluid sampling in the morning and evening.

3 rabbits each were randomly selected from the group of young, adolescent and adult animals, and were killed with an overdose of phenobarbital after joint fluid sampling. One of the knees in each animal was immediately removed and dissected. The femoral and tibial condyles were fixed in 10% formalin, decalcified in EDTA, embedded in paraffin, sectioned and stained with eosin-hematoxylin and Alcian blue-periodic acid Schiff.

To investigate a possible relation between joint fluid PG concentrations and knee cartilage mass, 18 additional adolescent (aged 18 *1.0* weeks) and 23 adult (35 *4.1* weeks) rabbits were used. After death, the cartilage was carefully removed with a scalpel from the knee joint surfaces and weighed.

Statistics

The volume of joint fluid, PG concentrations and cartilage masses did not differ between the right and left knees in all groups. We therefore combined the left and right knees and used the means for the statistical analyses. The paired t-test was used for intraanimal comparisons. ANOVA and post hoc tests were used for interanimal comparisons and the Pearson correlation to analyze the degree of relation between maturation and PG concentrations. To define the sensitivity and specificity of the analytic method, an arbitrary PG concentration of 3.9 μg/mL was employed to distinguish between young and adult rabbits.

Results

The histological sections showed no sign of degenerative change in the articular cartilage.

The amounts of aspirated joint fluid were similar in the three age groups (Table 1).

The PG concentrations in joint fluid of young, adolescent and adult rabbits differed significantly from one another (Table 1). The PG concentrations showed a moderate inverse correlation with maturation stage

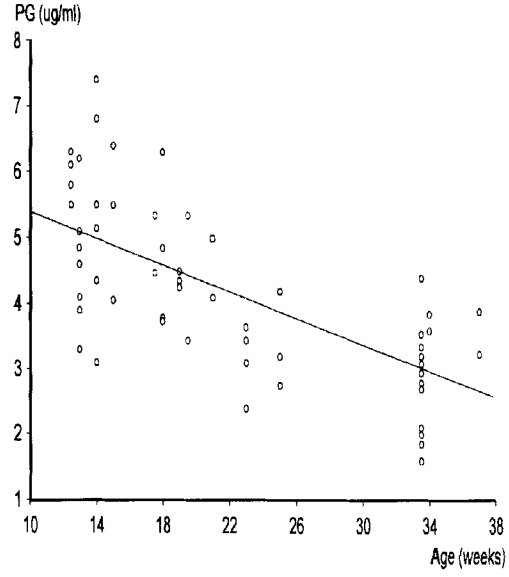

Figure 1. Correlation between the PG concentration in knee joint fluid and the age of the rabbits (r –0.69; p < 0.001).

(Figure 1). There was also a moderate inverse correlation between the PG concentrations and rabbit weight. Adolescent male rabbits had higher PG concentrations in joint fluid than adolescent female rabbits. PG concentrations were similar in morning and evening samples (Table 2). The sensitivity and specificity of the AB-method for distinguishing between young and adult rabbits were 91%. Adolescent (83 *20* mg/knee) and adult rabbits (77 *13* mg/knee) had similar cartilage masses. There was no correlation between age and cartilage mass.

Discussion

Under normal conditions, there is an equilibrium between synthesis and degradation of proteoglycans in articular cartilage and release to joint fluid. A recent

study suggested that patient age might not influence joint fluid PG concentrations (Dahlberg et al. 1992). However, only adult patients were investigated, who mostly had knee joint derangement; this series may therefore give no answer about the pure effect of age. Further, it is necessary to distinguish between age differences during the maturation process and changes during adult life. Our study clearly showed that the joint fluid PG concentrations decreased with increasing age during maturation, and this despite the use of equal amounts of joint dilution in all age groups, which may have the potential to reduce relatively the concentrations in younger, smaller joints with less genuine joint fluid. However, this maturation-dependent difference, of course, is not representative of the rest of the adult life span. The relatively high PG concentrations in young animals might be the result of a higher turnover rate in the growing articular cartilage. The effect of maturation on cartilage protein synthesis has been studied in rabbits, and it has been shown that synthetic activity is greatest at 2 months of age, but diminishes rapidly by 6 months to a rate which remains constant throughout adulthood (Mankin and Baron 1965). These results were confirmed by another study looking specifically at glycosaminoglycan turnover in rabbit articular cartilage (Maroudas 1975). Further, it has been shown that proteoglycan content in articular cartilage decreases with increasing age (Inerot and Heinegård 1978, Roughley and White 1980, Garg and Swann 1981). The changes in the PG concentrations in the present experiment agree with these results and thus may reflect the metabolic activity in articular cartilage during the process of maturation. This obvious maturation-dependent decrease in PG concentrations has resulted in a rather good distinction between young and adult animals, using this marker.

As decreasing cartilage mass in the arthrotic joint was associated with decreasing PG concentrations in joint fluid, it has been proposed that this marker is influenced by the amount of cartilage matrix remaining in the diseased joint (Dahlberg et al. 1992). However, in our material there seemed to be no relation between joint fluid PG concentrations and cartilage mass in healthy animals.

In a recent clinical study of adult patients with joint disease, no sex-dependent differences were demonstrated for this marker (Dahlberg et al. 1992). It may be speculated that joint disease which has been found to increase the PG concentration 3-fold in chronic cases (Lohmander et al. 1994) had a greater effect on this marker protein than a difference in sex, possibly masking such differences. Moreover, the sex-dependent differences demonstrated in our rabbit experiment may be more pronounced in adolescence, when specific hormonal activity is quite high, and may no longer be present in adults. A recent study demonstrated a higher proteoglycan synthesis rate and content in articular cartilage of male than of female adolescent rats (Larbre et al. 1994). It may be speculated that this higher synthetic activity also leads to a higher turnover and PG release into joint fluid, which could explain the sex-dependent differences found in the present study. However, possible size-differences between male and female joints may also have influenced the results. The male rabbit knee may have been somewhat larger and contained more genuine joint fluid than the female one, which would readily explain the higher PG concentrations in the former, especially since the amount of aspirated diluted samples were similar in both sexes.

Loading condition can influence the metabolism of articular cartilage. Long-term running exercise in dogs was paralleled by an increased synthesis and content of proteoglycans in articular cartilage (Paukkonen et al. 1985). However, walking exercise in dogs did not induce detectable changes in the proteoglycan synthesis of chondrocytes (Kincard and Van Sickle 1982). A study in human volunteers demonstrated a moderate increase in PG concentrations in the knee joint fluid after strenuous physical exercise and the authors therefore recommended some restriction in physical activity on the day of joint fluid sampling (Roos et al. 1993). The present results showed that cage activity did not change the joint fluid PG concentrations, suggesting that sampling during normal daily activity is reliable.

In conclusion, joint fluid PG concentrations depend on maturation stage and probably also on sex. Although physiologic variation seems to have less influence than diseases have, it should be taken into account when using this marker for joint diseases.

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References

Björsson S. Simultaneous preparation and quantitation of proteoglycans by precipitation with Alcian blue. Anal Biochem 1993; 210 (2): 282-91.

Dahlberg L, Ryd L, Heinegård D, Lohmander L S. Proteoglycan fragments in joint fluid. Influence of arthrosis and inflammation. Acta Orthop Scand 1992; 63 (4): 417-23.

Garg H G, Swann D A. Age-related changes in the chemical composition of bovine articular cartilage. Biochem J 1981; 193 (2): 459-68.

Inerot S, Heinegård D. Articular-cartilage proteoglycans in aging and osteoarthritis. Biochem J 1978; 169 (1): 143-56.

Kincard S A, Van Sickle D C. Effects of exercise on the histochemical changes of articular chondrocytes in adult dogs. Am J Vet Res 1982; 43 (7): 1218-26.

Larbre J P, Da Silva J A P, Moore A R, James I T, Scott D L, Willoughby D A. Cartilage contribution to gender differences in joint disease progression. A study with rat articular cartilage. Clin Exp Rheumatol 1994; 12 (4): 401-8.

Lohmander LS , Dahlberg L, Ryd L, Heinegård D. Increased levels of proteoglycan fragment in knee joint fluid after injury. Arthritis Rheum 1989; 32 (11): 1434-42.

Lohmander L S, Roos H, Dahlberg L, Hoerrner L A, Lark M. Temporal patterns of stromelysin-1, tissue inhibitor, and proteoglycan fragments in human knee joint fluid after injury to the cruciate ligament or meniscus. J Orthop Res 1994; 12 (1): 21-8.

Mankin H J, Baron P A. The effect of aging on protein synthesis in articular cartilage of rabbits. Lab Invest 1965; 14 (6): 658-64.

Maroudas A. Glycosaminoglycan turnover in articular cartilage. Philos Trans R Soc Lond (Biol) 1975; 271 (912): 293-313.

Messner K, Gillquist J, Björnsson S, Lohmander L S. Proteoglycan fragments in rabbit joint fluid correlated to arthrosis stage. Acta Orthop Scand 1993; 64 (3): 312-6.

Myers S, Brand K, Eilam O. Low-grade synovitis accelerates clearance of protein from the knee. Implications for measurement of synovial fluid "markers" in osteoarthritis. Trans Orthop Res Soc 1995; 20: 325.

Odenbring S, Egund N, Lindstrand A, Lohmander L S. Proteoglycan epitope in synovial fluid in gonarthrosis. Acta Orthop Scand 1991; 62 (2): 169-73.

Paukkonen K, Selkäinaho K, Jurvelin J, Kiviranta I, Helminen H J. Cells and nuclei of articular cartilage chondrocytes in young rabbits enlarged after non-strenuous physical exercise. J Anat 1985; 142 (1): 13-20.

Roos H, Dahlberg L, Lohmander L S. Proteoglycan fragments in knee joint fluid after exercise. Scand J Med Sci Sports 1993; 3 (1): 127-30.

Roughley P J, White R J. Age-related changes in the structure of the proteoglycan subunits from human articular cartilage. J Biol Chem 1980; 255 (1): 217-24.