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OSTEOARTHRITIS and CARTILAGE

Markers of cartilage matrix metabolism in human joint fluid and serum: the effect of exercise

By Harald Roos*, Leif Dahlberg*, Lori A. Hoerrner†, Michael W. Lark†, Eugene J.-M. A. Thonar‡, Masayuki Shinmei§, Ulla Lindqvist¶ and L. Stefan Lohmander*

**Department of Orthopaedics, University Hospital, S-221 85 Lund, Sweden; †Department of Biochemical & Molecular Pathology, Merck Research Laboratories, Rahway, New Jersey 07065, U.S.A.; ‡Departments of Biochemistry and Internal Medicine, Rush Medical College, Chicago, Illinois 60612, U.S.A.; §Department of Orthopedic Surgery, National Defense Medical College 3-2 Namiki, Tokorozawa, Saitama, 359 Japan; ¶Department of Clinical Chemistry, Academic Hospital, S-751 85 Uppsala, Sweden*

Summary

The concentrations of cartilage proteoglycan (aggrecan), stromelysin-1, tissue inhibitor of metalloproteinases-1 (TIMP-1) and procollagen II C-propeptide in knee joint fluid and the levels of aggrecan, hyaluronan and keratan sulfate in serum were measured before and after exercise in 33 healthy athletes. The samples before exercise were obtained after 24 h rest from running or soccer and the samples after exercise were obtained 30–60 min after the exercise. Nine athletes ran on a treadmill for 60 min, 16 ran on road for 80 min and 8 played one soccer game (90 min). A reference group of 28 patients with knee pain but not evidence of joint pathology or injury was used for comparison. In joint fluid no single marker from the degradative processes in cartilage matrix changed significantly with exercise but all showed a rising trend. All markers except stromelysin showed lower concentrations in athletes at rest compared to the reference group. In serum from runners before exercise the concentration of keratan sulfate was significantly higher than in both the soccer and reference groups and further increased after exercise. The increase in markers after exercise may reflect an effect of mechanical loading in combination with a possible high turnover rate of body cartilage matrix in these individuals.

Key words: Knee joint, Cartilage, Joint fluid, Serum, Markers, Exercise.

Introduction

In osteoarthritis (OA), the disease mechanisms active at the tissue and cell levels are unknown. The disease progress is related to degradation of joint cartilage matrix and loss of functional joint cartilage. Studies on experimental models of secondary OA have shown early changes in the metabolic, chemical and mechanical properties of the matrix before the appearance of radiological changes [1]. Injuries to the cartilage, menisci and ligaments of the knee often lead to post-traumatic OA [2–7]. These injuries are associated with a dramatic increase in the release of cartilage proteoglycan (aggrecan) fragments to the joint fluid [8]. The increased concentration of aggrecan fragments in the joint fluid reflects an increase in matrix turnover and could be related to the increased concentrations of metalloproteinases in joint fluid following trauma [9–11]. It has been further suggested that levels of keratan sulfate in serum may provide information on the systemic changes in metabolism of cartilage proteoglycans [12–15].

Occupational and sports-related joint loading may be important risk factors for OA [16–20]. Investigations on cartilage matrix metabolism in connection with joint loading would thus seem warranted. The purpose of the present study was to examine the effects of short-term physical exercise in humans on the release of markers of cartilage matrix turnover into knee joint fluid and serum.

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Address correspondence to: Harald Roos, Department of Orthopedics, University Hospital, S-22185, Lund, Sweden.

Materials and methods

A sample of blood and synovial fluid was obtained from 33 athletes (30 males) before and after exercise. There were three types of athletes with different training levels: recreational runners, mean age 32 years, who ran 5–10 km 2–3 times weekly; soccer players, with a mean age of 24 years, in the Swedish second league and a training level of 90 min 4 times weekly; and long distance runners and orienteerers, mean age of 31 years, with a weekly training dosage of 100–200 km of running. Orienteering is a sport in which competitors in the shortest possible time navigate unfamiliar terrain and locate checkpoints with the aid of a map and compass. The athletes were all healthy and without knee complaints. The first sample was taken at least one day before the planned exercise and at least one day after an ordinary training event. Three different types of physical exercise were performed. The recreational runners ($N = 9$) ran on a treadmill for 60 min, the soccer players ($N = 8$) played one soccer game (90 min) and the long distance runners ($N = 6$) and orienteerers ($N = 10$) ran 21 km (75–85 min) on road. A new sampling of synovial fluid and blood was done 30–60 min after the exercise. The athletes were compared to a reference group consisting of 28 patients (17 males), mean age 25 years, with chronic knee pain leading to an arthroscopy but with no recent knee injury or joint pathology diagnosed by arthroscopy, radiology or blood chemistry. Samples from these patients were obtained at the time of arthroscopy. All procedures were approved by the Ethics Review Board of the Medical Faculty, University of Lund.

ASPIRATION OF BLOOD

The blood samples were aspirated from the median antecubital vein, allowed to coagulate, centrifuged and the serum stored frozen in aliquots at −70°C until analyzed.

ASPIRATION OF KNEE JOINT

Joint fluid samples were aspirated by a lateral parapatellar approach, centrifuged, and aliquots of the supernatant frozen and stored in aliquots at −70°C within a few hours of collection.

AGGRECAN FRAGMENTS IN JOINT FLUID

Aggrecan fragments in joint fluid were quantified as sulfated glycosaminoglycan by precipitation with Alcian Blue, using chondroitin-6-sulfate as standard [21]. Values are expressed as μg sulfated glycosaminoglycan (aggrecan) per ml joint fluid. The values obtained with this assay represent aggrecan fragments in joint fluid [22] and show good correlation with a core protein epitope-based assay for aggrecan fragment in joint fluid [10, 23].

PROCOLLAGEN II C-PROPEPTIDE IN JOINT FLUID

The C-terminal propeptide of collagen type II was assayed by an ELISA with the use of rabbit antibodies raised against bovine procollagen-II C-propeptide immobilized on polystyrene balls [24]. Values are expressed as ng of procollagen II C-propeptide per ml joint fluid.

STROMELYSIN AND TISSUE INHIBITOR OF METALLOPROTEINASES (TIMP) IN JOINT FLUID

Stromelysin-1 and TIMP-1 concentrations in joint fluid were determined by sandwich ELISA using monoclonal and polyclonal antibodies raised against the human recombinant proteins, with the recombinant human proteins as standards [9, 25, 26]. The assay for stromelysin detects the proform of the enzyme, the large molecular weight active forms or enzyme complexed to TIMP but not to alpha-2-macroglobulin. The assay for TIMP-1 as used detects free TIMP-1 but not the inhibitor complexed with metalloproteinases. Molar ratios between stromelysin and TIMP were calculated with the assumption that the large majority (>90%) of stromelysin in joint fluid in the conditions examined in this study is present in the proform [9, 26]. Values are expressed as nM of stromelysin-1 or TIMP-1, respectively. Weight ratios between stromelysin and procollagen II C-propeptide were also determined in order to express the relation between potential degradation (stromelysin) and synthesis (procollagen II C-propeptide) in cartilage matrix.

AGGRECAN FRAGMENTS IN SERUM

Concentrations of aggrecan fragments in serum were determined by immunoassay with a polyclonal antibody preparation directed against the chondroitin sulfate-bearing domain of aggrecan [23]. Values are expressed as ng of intact aggrecan per ml serum.

ASSAY OF KERATAN SULFATE IN SERUM

Keratan sulfate in serum was measured by an ELISA using the 5-D-4 monoclonal antibody [27].

The levels are reported as equivalents of an international standard of keratan sulfate purified from human costal cartilage [12]. Values are expressed as ng per ml serum.

HYALURONAN IN SERUM

Serum hyaluronan was assayed by a commercial research radioassay (Pharmacia HA Test 50, Kabi Pharmacia Diagnostics, Uppsala, Sweden) [28]. The assay utilizes ^{125}I-labeled cartilage protein with affinity for hyaluronan and hyaluronan-substituted Microsepharose. The labeled protein was partitioned between the unknown amount of hyaluronan in serum and a fixed amount of hyaluronan bound to the Sepharose gel. The radioactivity was measured in the gel phase and compared with a standard curve. The reference range of hyaluronan in serum is 10–100 μg/l [29].

The statistical significance of differences was analyzed by nonparametric tests. The Wilcoxon Matched-Pairs Signed-Rank test was used to determine the differences before and after exercise. The Mann-Whitney rank-sum test for two independent samples was used to calculate differences between the athletes and the reference group. A two-tailed P-value of <0.05 was considered significant and all differences discussed as such are significant at this level or better.

Results

JOINT FLUID

Volume

The median volume of joint fluid aspirated before and after exercise was 1.0 ml, while the median volume for the reference group was 0.5 ml (Table I).

Aggrecan fragments

No significant difference in the concentration of sulfated glycosaminoglycans was found in athletes (62 μg/ml) as compared to the reference group (69 μg/ml) or in athletes before vs after exercise (Table I).

Procollagen II C-propeptide

The median concentration in the athletes (1.3 ng/ml) was lower than in the reference group (2.5 ng/ml) but was unchanged in the athletes after exercise (Table I).

Stromelysin-1

The median concentration of stromelysin was higher in athletes (6.6 nM) than in the reference

Table I
Markers of cartilage turnover in joint fluid and serum

	Athletes before	Athletes after	Reference*	Statistics†		
				B/A	B/R	A/R
Synovial Fluid						
Volume‡	0.9 (0.1–5.5) [17]	1.0 (0.1–10.0 [20]	0.5 (0.01–5.0) [28]	NS [9]	NS	NS
AGN§	62 (5–107) [12]	74 (8–213) [17]	69 (32–99) [26]	NS [6]	NS	NS
pCol-II-C¶	1.5 (0.4–3.4) [8]	1.2 (0.1–5.7) [15]	2.5 (0.9–8.2) [16]	NS [6]	S	S
SLN‖	6.6 (0.2–49.1) [12]	12.0 (0.2–37.5) [20]	4.3 (0.2–42.5) [25]	NS [7]	S	S
TIMP‖	5.0 (0.5–15.6) [12]	6.1 (0.5–13.7) [20]	5.4 (1.9–15.9) [25]	NS [8]	NS	NS
SLN/TIMP**	1.1 (0.3–7.9) [12]	2.0 (0.1–6.1) [20]	0.97 (0.03–3.01) [25]	NS [7]	S	S
SLN/pC-II-C††	6.8 (1.0–30.7) [8]	3.8 (0.5–22.3) [15]	2.2 (0.1–12.2) [16]	NS [6]	S	S
Serum						
KS‡‡	311 (154–500) [32]	329 (154–590) [31]	252 (156–414) [27]	S [29]	S	S
AGN§§	275 (110–360) [12]	280 (70–460) [20]	220 (60–400) [26]	NS [9]	NS	NS
HA[1]¶¶	21.8 (0–35.3) [12]	24.7 (11–51.3) [20]	16.8 (11.1–30.9) [27]	NS [8]	NS	S

The values are expressed as: median (range) [N].
*Reference group consisting of 28 subjects with knee pain but normal arthroscopy, radiography and laboratory tests.
†Only paired samples were used for the statistical calculation of the difference before and after exercise. B/A = Before vs After exercise, B/R = Before exercise vs Reference group, A/R = After exercise vs Reference group. S = P-value <0.05. NS = P-value >0.05.
‡Volume of aspirated joint fluid (ml).
§Aggrecan fragments measured as sulfated glycosaminoglycan (μg/ml).
¶Procollagen II C-propeptide(ng/ml).
‖Stromelysin-1 and TIMP-1, respectively (nM).
**Molar ratio of stromelysin to TIMP.
††Weight ratio of stromelysin to procollagen II C-propeptide.
‡‡Keratan sulfate (ng/ml).
§§Aggrecan fragments measured as aggrecan core protein epitope (ng/ml).
¶¶Hyaluronan (μg/ml).

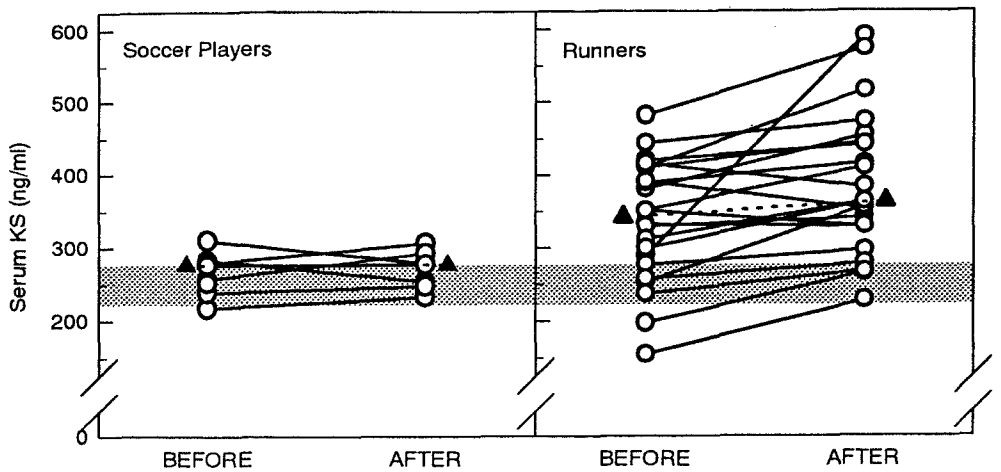

FIG. 1. Concentration of keratan sulfate in serum. Left: soccer players, right: runners before and after exercise. Median values for each respective group are given (▲---▲). The shaded zone indicates the 25th and 75th percentiles of the reference group.

group (4.3 nM) but no difference was seen between the athletes before and after exercise (Table I).

TIMP-1

No differences in median concentrations were found between athletes (5.0 nM) and the reference group (5.4 nM) or in athletes before or after exercise (Table I).

Molar ratio stromelysin/TIMP

The molar ratio of stromelysin to TIMP was increased in the athletes (1.1) compared to the reference group (0.97) but showed no change after exercise (Table I).

Weight ratio stromelysin/procollagen II C-propeptide

The weight ratio of stromeylsin to procollagen II C-propeptide differed in the athletes (6.8) compared to the reference group (2.2) but exercise did not change the ratio (Table I).

SERUM

Aggrecan fragments

The median concentration of aggrecan fragments in serum was higher in athletes (275 ng/ml) than in the reference group (220 ng/ml) but exercise did not influence the concentration. The values before or after exercise did not alone differ from the reference group (Table I).

Keratan sulfate

The median concentration before exercise was 311 ng/ml compared to 329 ng/ml after exercise and the paired test showed a significant difference. The concentration before and after exercise showed a higher median concentration than in the reference group (252 ng/ml) (Table I and Fig. 1). In the reference group the median concentration serum keratan sulfate from males (270 ng/ml) was higher than in females (232 ng/ml).

Since we found an increased concentration of keratan sulfate in serum in the athletes compared with the reference group, we decided to investigate the effect of the amount of exercise on serum levels of keratan sulfate. The group of athletes was thus divided into soccer players ($N = 7$ with available samples before and after exercise) and runners ($N = 24$). A paired analysis of serum concentrations of keratan sulfate in the soccer players before and after exercise showed no difference due to exercise. In addition, the median concentration of keratan sulfate in the soccer players did not differ from the reference group [Fig. 1(a)]. However, similar analysis of the samples from the runners showed that at rest they had a higher median concentration than both the reference group and the soccer players (Table II). A further paired comparison within the runner group showed an increase due to exercise [Table II, and Fig. 1(b)].

Hyaluronan

The median concentration before exercise in athletes was 21.8 μg/l and after exercise it was 24.7 μg/l. The value after exercise differs from the

reference value of 16.8 μg/l. There was no difference between runners and soccer players or between values before and after exercise (Tables I and II).

Discussion

A previous study showed a significant increase of aggrecan core protein epitopes in paired samples after exercise [30]. In the present study we have chosen to analyze joint fluid components that are suggested to represent both cartilage matrix degradation (aggrecan fragments, stromelysin and TIMP) and synthesis (procollagen II C-propeptide). We also compare the findings in joint fluid with serum values for aggrecan. In addition, we study the serum levels of keratan sulfate, which are suggested to mirror systemic body cartilage degradation [13, 31]. Hyaluronan in serum was shown in a previous study to be related to the level of physical activity [32].

Several different factors may influence the concentrations of cartilage markers in synovial fluid and the relevance of the different marker levels are not yet clearly understood. An increased release of a marker may be caused by either a higher turnover rate of the matrix or by an increased degradation with a net loss of the marker from the matrix to the joint fluid. The marker levels are dependent upon both the rate of release from the matrix into the joint fluid and the rate of removal from the joint compartment [33, 34]. In synovial fluid the matrix molecules may be further degraded by the synovial cells or removed by bulk flow to the lymph circulation. The half-life of aggrecan and hyaluronan in the joint fluid has been estimated to be 12–18 h [35]. The degradation products from the matrix are further degraded in the lymph nodes before they reach the blood, from which they are eliminated via the liver and kidneys [36]. The half-life of hyaluronan in circulation is very short (a few minutes) [32] and that of keratan sulfate some 5–50 min [37].

The levels of aggrecan, stromelysin and TIMP showed no significant differences before and after exercise when analyzed separately, but all three markers, which have been suggested to represent degradative processes in cartilage matrix, showed a rising trend with exercise. This may suggest that, taken as a group, the concentrations of these markers increase as a result of short-term physical exercise. In contrast, procollagen II C-propeptide, which is the product of processing of newly synthesized collagen type II released from the chondrocytes, did not rise in joint fluid concentration as a result of exercise. Moreover, the levels were lower in the athletes when compared with the reference group. The calculation of ratios between different markers avoids the confounding influence of an increased rate of synovial fluid production during exercise [33]. The increased ratio between stromelysin, representing a potential for cartilage degradation, and procollagen II C-propeptide, representing collagen synthesis, among the athletes may be consistent with a higher joint cartilage degradative activity compared to the reference group.

It is conceivable that the release of markers from the matrix is augmented by mechanical loading [30, 38]. Such an effect, alone or in combination with a higher turnover rate of the matrix in athletes, can explain the slight increase of aggrecan,

Table II
Serum markers in runners and soccer players

	Keratan sulfate*	Aggrecan	Hyaluronan
Before exercise			
Runners	351 (154–500) [24]	250 (110–360) [9]	17.8 (0–35.3) [9]
Soccer players	266 (217–310) [8]	280 (70–360) [3]	22.3 (22.1–34.3) [3]
After exercise			
Runners	359 (227–590) [24]	280 (100–460) [15]	26.3 (11.2–51.3) [15]
Soccer players	277 (232–307) [7]	265 (240–290) [5]	17.7 (11.0–33.4) [5]
Before vs after			
Runners	S [24]	NS [7]	NS [6]
Before vs after			
Soccer players	NS [7]	NS [2]	NS [2]
Runners vs soccer players			
back exercise	S	NS	NS
Runners vs references			
before exercise	S	NS	NS
Soccer players vs references			
before exercise	NS	NS	NS

*See Table I footnotes.

stromelysin and TIMP in joint fluid after exercise. An increase in trans-synovial transport from the joint cavity induced by an increased net filtration pressure could be caused by exercise [39] and the true release of markers could thus be higher than indicated here.

The aggrecan fragments were measured as chondroitin sulfate by dye precipitation in the present study [21] and the results of this method were shown to be in agreement with those found by immunoassay [10, 23]. However, it is notable that in joint fluid obtained from patients some time after a knee injury the levels of aggrecan fragments measured by dye precipitation are lower than if measured by ELISA [10]. The reasons for this discrepancy are not clear, but could explain the lack of increase in aggrecan fragments after exercise in the present study, compared to our previous study where the levels of aggrecan fragments were quantified by the immunoassay method [30].

The reference group used here included patients with chronic knee pain leading to arthroscopy, but where careful examination including arthroscopy, radiology and blood chemistry failed to reveal any joint-related pathology. Nevertheless, this group could include some individuals with undiagnosed joint disease. It is therefore not surprising that, in general, median joint fluid cartilage marker concentrations were somewhat higher in this group than in the knee-healthy athletes before exercise. For lack of a large group of samples from age-matched knee-healthy, nonexercising volunteers, we regard the present reference group as an acceptable substitute. Joint fluid from the contra-lateral knee of unilaterally knee-injured patients is not suitable as reference in studies such as these, since the cartilage matrix metabolism has been shown to be changed in the uninjured knee as determined by joint fluid markers [40].

There was no correlation between the concentrations of the markers in joint fluid and serum or between the different serum markers, in conformity with previous findings [31]. Serum concentrations of keratan sulfate vary with age but after the age of 15 show little change over a period of two or three years [31]. Several reports have shown that keratan sulfate concentrations in serum are elevated in OA [12, 13, 31], but other studies have failed to confirm this [41]. The average serum concentration of keratan sulfate in our reference group is similar to that of other populations without OA [12, 13], while the serum concentration in patients with hip OA [12, 13] and knee OA [31] was higher than in the athletes in this study.

The runners had higher serum concentrations of keratan sulfate at rest when compared to both soccer players and the reference group. After exercise the concentration increased further in these individuals, a finding which is in contrast to previous reports [15, 27, 42]. On the other hand, the levels of keratan sulfate in serum at rest and after exercise in the soccer players were about the same and did not differ from the reference group. In the reference group, males had higher serum keratan sulfate levels than females, similar to one previous report [31], but in contrast to another [12]. Due to a disproportion of males between the reference group and the athletes in the present study, a gender-matched comparison was performed. However, this did not eliminate the difference between the runners and the reference group. Several studies suggest that most keratan sulfate-bearing molecules in blood represent products of the degradation of cartilage proteoglycans [14, 15]. Only a small proportion of the body cartilage mass is, however, located in the joints, with the rest being found in the ribs, airways, intervertebral discs, etc. [43]. A marker present in joint fluid will be representative for the cartilage turnover in a single joint while markers present in serum will represent an integrated measure of turnover activity in all joints and possibly all body cartilage [44]. Keratan sulfate in serum is perhaps one example of a cartilage marker which may serve as an indicator of general body cartilage mechanism [13] and the results in this study may thus reflect a higher cartilage turnover among the runners than the other groups. Changes in the matrix composition of joint cartilage and intervertebral discs in beagle dogs after a long period of vigorous running have been shown, possibly reflecting an adaptation to enhanced load [45, 46]. The individuals in the group of runners train very hard and could thus exercise their tracheobronchial-, costal- and joint cartilage, as well as intervertebral discs. The difference in keratan sulfate levels in serum between the soccer players and runners could be an effect of different physical activity in the two sports.

No significant effect of exercise was noted on serum levels of hyaluronan or aggrecan fragments. The increased values of hyaluronan among the athletes compared to the reference group, consistent with previous findings on the relation of hyaluronan to activity level [47], may be due to the athletes as a group being more physically active. Since the post-exercise samplings were performed 30–60 min after the exercise, the short half-life of hyaluronan in serum could explain the unchanged values after exercise, which is in contrast to the previously cited study [47].

There is no clear evidence that the moderate changes in cartilage markers in joint fluid and serum shown here to result from physical exercise reflect a harmful effect on joint cartilage. The most reliable explanation is probably that the higher cartilage turnover rate is responsible for the slight increases in marker concentrations among the athletes. Published reports on cartilage markers in joint fluid and serum frequently lack control groups; the results presented here could thus serve as a useful reference for continued work on these markers.

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References

1. Carney SL, Billingham MEJ, Muir H, Sandy JD. Demonstration of increased proteoglycan turnover in cartilage explants from dogs with experimental osteoarthritis. *J Orthop Res* 1984;**2:**201–6.
2. Fairbank TJ. Knee joint changes after meniscectomy. *J Bone Joint Surg [Br]* 1948;**30:**664–70.
3. Johnson RJ, Kettelkamp DB, Clark W, Leaverton PF. Factors affecting late results after meniscectomy. *J Bone Joint Surg [Am]* 1974;**56:**719–29.
4. Noyes FR, Mooar PA, Matthews DS, Butler DL. The asymptomatic anterior cruciate-deficient knee. *J Bone Joint Surg [Am]* 1983;**65:**154–62.
5. Kannus P, Järvinen M. Post-traumatic anterior cruciate ligament insufficiency as a cause of osteoarthritis in a knee joint. *Clin Rheumatol* 1988;**8:**251–60.
6. Sherman W, Warren RF, Marshall J, Savatsky GJ. A clinical and radiological analysis of 127 anterior cruciate insufficient knees. *Clin Orthop* 1988;**227:**229–37.
7. Roos H, Adalberth T, Dahlberg L, Lohmander LS. Post-traumatic osteoarthrosis of the knee: disease progress after injury to cruciate ligament or meniscus. *Trans Orthop Res Soc* 1994;**19:**218.
8. Lohmander LS, Dahlberg L, Ryd L, Heingegård D. Increased levels of proteoglycan fragment in knee joint fluid after injury. *Arthritis Rheum* 1989;**32:**1434–42.
9. Lohmander LS, Roos H, Dahlberg L, Hoerrner LA, Lark MW. Temporal patterns of stromelysin, tissue inhibitor and proteoglycan fragments is synovial fluid after injury to knee cruciate ligament or meniscus. *J Orthrop Res* 1994;**12:**21–8.
10. Lohmander LS, Hoerrner LA, Dahlberg L, Roos H, Björnsson S, Lark MW. Stromelysin, tissue inhibitor and proteoglycan fragments in human knee joint fluid after injury. *J Rheumatol* 1993;**20:**1362–8.
11. Lohmander LS, Hoerrner LA, Lark MW. Metalloproteinase, tissue inhibitor and proteoglycan fragments in knee synovial fluid in human osteoarthritis. *Arthritis Rheum* 1993;**36:**181–9.
12. Thonar EJ-MA, Lentz ME, Klintworth GK *et al.* Quantification of keratan sulphate in blood as a marker of cartilage metabolism. *Arthritis Rheum* 1985;**28:**1367–76.
13. Sweet MBE, Coelho A, Schnitzler CM *et al.* Serum keratan sulphate levels in osteoarthritis patients. *Arthritis Rheum* 1988;**31:**648–52.
14. Williams JM, Downey C, Thonar EJ-MA. Increase in levels of serum keratan sulphate following cartilage proteoglycan degradation in the rabbit joint. *Arthritis Rheum* 1988;**31:**557–60.
15. Block JA, Schnitzer TJ, Anderson GBJ *et al.* The effect of chemonucleolysis on serum keratan sulphate levels in humans. *Arthritis Rheum* 1989; **32:**100–4.
16. Klünder K, Rud B, Hansen J. Osteoarthritis of the hip and knee joint in retired football players. *Acta Orthop Scand* 1980;**51:**925–7.
17. Lindberg H, Roos H, Gärdsell P. The prevalence of coxarthrosis in former soccer players. *Acta Orthop Scand* 1993;**64:**165–7.
18. Marti B, Knobloch M, Tschopp A, Jucker A, Howald H. Is excessive running predictive of degenerative hip disease? Controlled study in former elite athletes. *BMJ* 1989;**299:**91–3.
19. Roos H, Lindberg H, Gärdsell P, Lohmander LS, Wingstrand H. The prevalence of gonarthrosis and its relation to meniscectomy. *Am J Sports Med* 1994;**22:**219–21.
20. Vingård E, Alfredsson L, Goldie I, Hogstedt C. Sports and osteoarthrosis of the hip. An epidemiological study. *Am J Sports Med* 1993;**21:**195–200.
21. Björnsson S. Simultaneous preparation and quantification of proteoglycans by precipitation with Alcian Blue. *Anal Biochem* 1993;**210:**282–91.
22. Björnsson S. Size dependent separation of proteoglycans by electrophoreses in gels of pure agarose. *Anal biochem* 1993;**219:**292–8.
23. Saxne T, Heinegård D, Wollheim FA. Cartilage proteoglycans in synovial fluid and serum in patients with inflammatory joint disease. *Arthritis Rheum* 1987;**30:**972–9.
24. Shinmei M, Ito K, Matsuyama S, Yoshihara Y, Matsuzawa K. Joint fluid carboxy-terminal type II procollagen peptide as a marker of cartilage collagen biosynthesis. *Osteoarthritis Cart* 1993;**1:**121–8.
25. Cooksley S, Hipkiss JB, Tickle SP *et al.* Immunoassays for the detection of human collagenase, stromelysin; tissue inhibitor of metalloproteinases (TIMP) and enzyme inhibitor complexes. *Matrix* 1990;**10:**285–91.
26. Walakovits LA, Bhardvaj N, Gallick GS, Lark MW. Detection of high levels of stromelysin and collagenase in synovial fluid from patients with rheumatoid arthritis and post-traumatic knee injury. *Arthritis Rheum* 1992;**35:**35–42.
27. Thonar EJ-MA, Williams JM, Maldonado BM *et al.* Serum keratan sulphate concentration as a measure of a catabolism of cartilage proteoglycans, monoclonal antibodies, cytokines, and arthritis. In Kressina TF, Ed. *Mediators of Inflammation and Therapy.* New York: Marcel Decker 1991.
28. Brandt R, Hedlöf E, Åsman I, Bucht A, Tengblad A. A convenient radiometric assay for hyaluronan. *Acta Otolaryngol (Stockh)* 1987;(suppl 442):31–5.

29. Engström-Laurent A, Laurent UBG, Lilja K, Laurent TC. Concentration of sodium hyaluronate in serum. *Scand J Clin Lab Invest* 1985; **45:**497–504.
30. Roos H, Dahlberg L, Lohmander LS. Proteoglycan fragments in knee joint fluid after exercise. *Scand J Med Sci Sports* 1993;**3:**127–30.
31. Campion GV, McCrae F, Schnitzer TJ, Lenz ME, Dieppe PA, Thonar EJ-MA. Levels of keratan sulfate in the serum and synovial fluid of patients with osteoarthritis of the knee. *Arthritis Rheum* 1991;**34:**1254–9.
32. Laurent TC, Fraser RE. Hyaluronan. *FASEB J* 1992;**6:**2397–404.
33. Levick JR. The clearance of macromolecular substances such as cartilage markers from synovial fluid and serum. In Maroudas A, Kuettner K, Eds. *Methods in Cartilage Research.* London: Academic Press 1990:352–62.
34. Levick JR. Flow through interstitium and other fibrous matrices. *Q J Exp Physiol* 1987;**72:**409–37.
35. Page-Thomas DP, Bard D, King B *et al.* Clearance of proteoglycan from joint cavities. *Ann Rheum Dis* 1987;**46:**934–7.
36. Engström-Laurent A, Hellström S. The role of liver and kidneys in the removal of circulating hyaluronan, an experimental study in the rat. *Connect Tissue Res* 1990;**24:**219–24.
37. Maldonado BA, Williams JM, Otten LM, Flannery M, Kuettner KE, Thonar EJ-MA. Differences in the rate of clearance of different KS-bearing molecules injected intravenously in rabbits. *Trans Orthop Res Soc* 1989;**14:**161.
38. Sah RL-Y, Doong J-YH, Grodzinsky AJ *et al.* Effect of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants. *Arch Biochem Biophys* 1991;**286:**20–9.
39. Levick JR. Blood flow and mass transport in synovial joints. In Renkin EM, Michel CC, Eds. *Handbook of Physiology—The Cardiovascular System IV, Microcirculation part* 2. Bethesda, MD: American Physiological Society 1984:917–47.
40. Dahlberg L, Roos H, Saxne T, Heinegård D, Lark M, Lohmander LS. Markers of cartilage metabolism in joint fluid in the injured and uninjured knee of the same patient. *Trans Orthop Res Soc* 1994;**19:**261.
41. Spector TD, Woodward GM, Hall GM *et al.* Keratan sulphate in rheumatoid arthritis, osteoarthritis, and inflammatory diseases. *Ann Rheum Dis* 1992;**51:**1134–7.
42. Sweet MBE, Jakim I, Coelho A, Becker PJ, Thonar EJ-MA. Serum keratan sulfate levels in marathon runners. *Int J Sports Med* 1992;**13:**358–350.
43. Atencia LJ, McDevitt CA, Nile WB, Sokoloff L. Cartilage content of an immature dog. *Connect Tissue Res* 1989;**18:**235–42.
44. Lohmander LS. Osteoarthritis: man models and molecular markers. In: Maroudas A, Kuettner K, Eds. *Methods in Cartilage Research.* London: Academic Press, 1990:337–40.
45. Säämänen A-M, Puustjärvi K, Ilves K *et al.* Effect of running exercise on proteoglycans and collagen content in the intervertebral disc of young dogs. *Int J Sports Med* 1993;**14:**48–51.
46. Kiviranta I, Tammi M, Säämänen A-M, Helminen HJ. Moderate running exercise augments glycosaminoglycans and thickness of articular cartilage in the knee joint of young Beagle dogs. *J Orthop Res* 1988;**6:**188–95.
47. Engström-Laurent A, Hällgren R. Circulating hyaluronic acid levels vary with physical activity in healthy subjects and in rheumatoid arthritis patients. *Arthritis Rheum* 1987;**30:**1333–8.