Adaptation of canine femoral head articular cartilage to long distance running exercise in young beagles

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Abstract
Objective—To study the effects of long term (one year), long distance (up to 40 km/day) running on the metabolism of articular cartilage the biosynthesis of proteoglycans was examined by in vitro labelling of anterior (weight bearing) and posterior (less weight bearing) areas of the femoral head from young beagles.
Methods—Total sulphate incorporation rates were determined and distribution of the incorporated sulphate was localised by quantitative autoradiography. Concentration and extractability of the proteoglycans were determined, and proteoglycan structures were investigated by gel filtration chromatography, agarose gel electrophoresis, and chemical determinations.
Results—In the less weight bearing area the amount of extractable proteoglycans was decreased (p≤0·02), simultaneously with an increased concentration of residual glycosaminoglycans in the tissue after 4 M GuCl extraction (p≤0·05). In control animals proteoglycan synthesis was most active in the deep zone of the cartilage, whereas exercise increased synthesis in the intermediate zone. There was a tendency to a lower keratan:chondroitin sulphate ratio in the running dogs. No macroscopical or microscopical signs of articular degeneration or injury were visible in any of the animals.
Conclusion—The articular cartilage of the femoral head showed a great capacity to adapt to the increased mechanical loading. The reduced proteoglycan extractability in the less weight bearing area changed it similar to the weight bearing area, suggesting that the low extractability of proteoglycans reflects the long term loading history of articular cartilage. The congruency of the femoral head with acetabulum seems to protect the cartilage from the untoward alterations that occur in the femoral condyles subjected to a similar running programme.

The continuous wellbeing of articular cartilage is vital for the joint as it is almost totally unable to recover from trauma. Weight bearing has been shown to be important for the main-tenance of normal articular cartilage, because amputation of the leg below the knee (allowing free movement of the joint) could not maintain normal composition of the cartilage.[1] Physical training has often been considered to have beneficial effects on the properties of articular cartilage, although catabolic responses to heavy exercise have also been reported.[2][3] An atrophied cartilage was not able to recover if running exercise was started immediately after immobilisation.[4]

Loading of an instable joint causes severe changes in the structure and function of articular cartilage.[5] Cutting of the anterior cruciate ligament or meniscectomy[6–9] leads to the appearance of osteoarthritic lesions. It has been suggested that the onset of osteoarthritis is related to increased turnover of proteoglycans.[8] Besides erosion and fibrillation in the tibial joints, meniscectomy causes enhanced extractability of proteoglycans in the femoral head, which was thought to be due to the deficiency of hyaluronan in the tissue.[10]

In our earlier experiments with animal models we have studied the role of immobilisation, remobilisation, and running exercise on the health and maintenance of normal articular cartilage.[11] These studies showed that the effects of immobilisation varied in different articular areas. In general, immobilisation leads to a remarkable local loss of proteoglycans from the matrix. Remobilisation caused the concentrations to revert to normal but not in all of the joint surfaces.[12][13] On the other hand, moderate running (4 km/day) increased proteoglycan content and enhanced the biomechanical properties of the cartilage.[14][15] These changes, regarded as positive, were partially lost in another running experiment (20 km/day), apparently owing to the higher degree of local strain and weight bearing.[16]

Whether a long distance running exercise (40 km/day, 15 weeks) affects the rate of proteoglycan synthesis was studied here in the canine femoral head. The incorporation rate of sulphate into articular cartilage was measured by in vitro labelling. The distribution of synthesis within the tissue was assessed by quantitative autoradiography. The specimen was divided into a weight bearing (anterior part) and a less weight bearing (posterior part) area to study possible differences due to weight bearing. The structure of the proteoglycan molecules was further characterised by biochemical analyses.

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Materials and methods

ANIMALS

Female beagle dogs of pure breed were provided by the National Laboratory Animal Centre (Kuopio, Finland) and by Shamrock Ltd (Hereford, UK). The dogs lived in standard cages with a floor area of 0.9×1.2 m (height 77 cm), according to National Institute of Health recommendations. For each experimental animal (n=10) a control of the same age was chosen from the same litter.

EXERCISE PROTOCOL

The dogs started the running exercise at the age of 15 weeks on a treadmill adjusted to 15° uphill inclination. The daily running distance was increased gradually, so that at the age of 55 weeks it was 40 km/day at a speed of 6 km/h (fig 1). The animals were killed at the age of 70 weeks.

SAMPLE PREPARATION

The dogs were killed with an overdose of barbiturate and the femoral head was dissected free of muscles with special care taken not to wound the cartilage surface. A dentist's drill equipped with two cutting discs and a 1 mm spacer was used to produce three cartilage slices. The slices were divided into anterior (weight bearing) and posterior (less weight bearing) sectors by sawing a cutting line horizontally at the superior part of the joint. The sampling method was chosen based on reported results of the directions of measured maximum hip joint forces in dogs with instrumented endoprosthesis.[17] The cited reference showed that the main loads acted at medioventral directions. The samples were removed from the femoral head, placed into culture dishes filled with ice cold Eagle's medium (EMEM, Flow Laboratories, Irvine, Scotland), supplemented with Earle's salts and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin), and transported to the tissue culture laboratory on an ice bath.

CHEMICAL ANALYSIS

Proteoglycans were quantified as uronic acid[18] and elution of proteoglycans or glycosaminoglycans in chromatographic runs was assayed by the Safranin O precipitation method[19] and its autoradiographic application to quantify macromolecular, radiolabelled glycosaminoglycans.[20] Concentration of DNA was determined from a proteinase K digest with calf thymus DNA as the standard.[21]

INCORPORATION OF ^{35}S-SULPHATE

One batch of ^{35}S-sulphate was diluted and used through the whole experiment (Amersham International, Little Chalfont, UK). The slices of explants were transferred into ice cold, fresh EMEM supplemented with Earle's salts and antibiotics. The tissue was preincubated for one hour at 4°C to ensure even distribution of the radioactive isotope precursor (1.8 MBq/ml) in tissue before labelling at 37°C in a 5% CO_2 atmosphere for four hours. Aliquots were taken to measure the radioactivity of the labelling medium. The synthetic activity was stopped by replacing the medium with ice cold phosphate buffered saline. One tissue block, containing the underlying bone, was cut from each sample for quantitative autoradiography and cartilage from the rest of the tissue (for measurement of sulphate incorporation) was dissected free from the bone and its wet weight was determined.

QUANTITATIVE AUTORADIOGRAPHY OF TISSUE SECTIONS

In vitro labelled tissue blocks (about 1 mm^3) were prepared for the autoradiography as described earlier[22] modified by an additional step for decalcification (4.13% EDTA in 0.1 M phosphate buffer, pH 7.4, for two days at 4°C). The grain density in developed tissue sections was assessed by image analysis. A colour CCD video camera WV-CD 132 (Panasonic, with 574×581 pixel resolution) was connected to a Leitz microscope (Wetzlar, Germany) and 40 images (total magnification × 1730 on the monitor) captured by QuickCapture DT 2255 frame grabber (Data Translation, Marlboro, MA) were averaged by image analysis software (Image; public domain software by Wayne Rasband, NIH) on a Macintosh fx microcomputer (Apple, Cupertino, CA) to produce the primary image. The grains concentrated in the centres of cells had to be thresholded into a separate binary image as the succeeding filtering excluded them. The primary image was then smoothed by a median filter, convoluted by a 5×5 high pass filter, and eroded before thresholding into another binary image. These binary images were summed by a logical OR operator to give the final image showing the pixels covered by grains. Each microscopical view was divided into three fields of the same size (107 μm width × 34 μm height respectively) and the relative proportion of total grain area in each field was counted. Consecutive views (four to five) starting from the superficial zone

Running schedule
At maximum 40 km/day
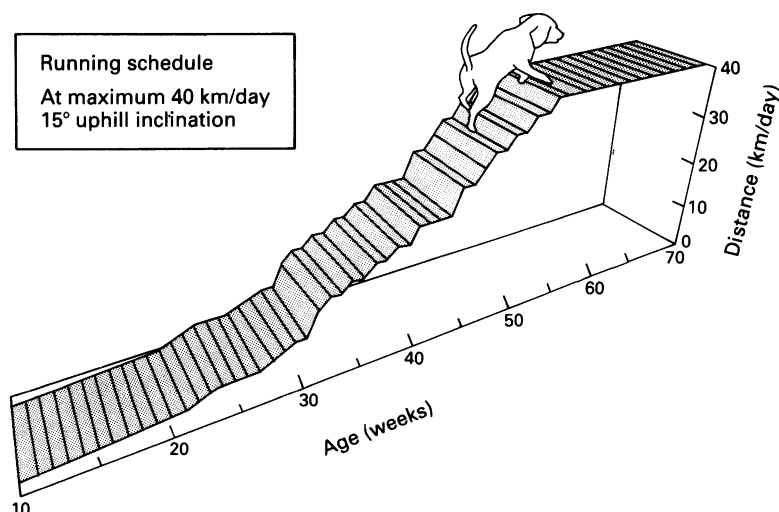15° uphill inclination

Figure 1　Schematic presentation of the running programme. After an adaptation period the 10 dogs ran daily (five days a week) 40 km/day with 15° uphill inclination for 15 weeks.

and ending in the deep zone were captured and quantified. For each animal, six sections were analysed.

MEASUREMENT OF SULPHATE INCORPORATION

Incorporation rates were estimated from proteinase K (type XXVIII, Sigma, St Louis, MO) digests of cartilage. Tissue pieces were digested for 17 hours at 60°C by 0·05% proteinase K in 0·1 M phosphate buffer (pH 7·4), 10 mM EDTA included. Aliquots of digests were eluted through Sephadex G-25 gel filtration columns (PD-10, Pharmacia, Uppsala, Sweden) to remove unincorporated sulphate precursor and radioactivity was measured by liquid scintillation (LKB, Bromma, Sweden) in duplicate aliquots from PD-10 eluates and from medium samples dissolved in a water soluble scintillation cocktail (OptiPhase, Pharmacia, Turku, Finland).

SULPHATE INCORPORATION INTO KERATAN SULPHATE

The sulphate incorporation into keratan sulphate was estimated by gel chromatographic separation of glycosaminoglycan chains and disaccharides produced after specific cleavage of chondroitin sulphate by chondroitinase ABC. Proteinase K digested samples, separately pooled in each group, were transferred into deionised water by PD-10 desalting columns and reduced with alkaline borohydride (1 M NaBH$_4$ in 0·05 M NaOH for 17 hours at 45°C). The samples were neutralised, lyophilised, and digested for six hours by chondroitinase ABC (Seikakagu Co, Tokyo, Japan) in 0·1 M TRIS-acetate, pH 8·0. The enzyme was heat denatured, the samples were chromatographed on a Sephacryl S-300 column (0·5×20 cm, eluted with 0·1 M sodium phosphate, pH 7·0, 0·5% CHAPS, 0·15 M NaCl, and 10 mM Na$_2$SO$_4$) and the fractions were analysed by liquid scintillation and Safranin O to determine the radioactivity in the macromolecular keratan sulphate and disaccharides produced from chondroitin sulphate.

EXTRACTION OF PROTEOGLYCANS

Cartilage was sliced into small pieces and proteoglycans were extracted with 4 M GuCl (Fluka, Buchs, Switzerland) in 50 mM sodium acetate, pH 5·8, including the bacterial growth and protease inhibitors: 10 mM disodium EDTA (Sigma), 100 mM ε-amino-n-caproic acid (Sigma), 5 mM benzamidine HCl (Sigma), and 0·02% sodium azide (w/v; Merck, Darmstadt, Germany). Extraction was continued for 60 hours.

The non-extractable fraction was digested by 0·05% proteinase K as described and transferred into deionised water by a desalting column and the residual uronic acid content was measured. The samples in each group were pooled and the relative glycosaminoglycan chain lengths were estimated by Sephacryl S-300 gel chromatography after keratanase and

chondroitinase ABC digestions as described later.

SODIUM DODECYL SULPHATE (SDS) AGAROSE GEL ELECTROPHORESIS OF THE PROTEOGLYCANS

Extracted proteoglycans were transferred into associative conditions by PD-10 gel chromatography (50 mM sodium acetate, pH 5·8, including the same inhibitors as described previously) and precipitated in 75% ethanol. Each sample was run in duplicate by agarose electrophoresis as described earlier.[13] Relative mobilities were compared with a chondroitin sulphate standard (from shark cartilage; Sigma) and bovine articular cartilage A1 proteoglycan, applied into the same gel.

The fixed and stained gels were dried on Gel-Bond and scanned with a grey-scale scanner (256 shades of grey) connected to a Macintosh fx microcomputer (Apple). The scanned images of the stained gels were quantified by image analysis software (Image). Autoradiography films exposed to gels for two weeks were developed for five minutes (DL-9, Eastman Kodak, Rochester, NY), washed for two minutes in water, then fixed for 11 minutes (AL-4, Eastman Kodak) followed by a wash under tap water. The films were then scanned and mean film density of the lanes was measured by image analysis.

SEPHACRYL S-500 GEL FILTRATION CHROMATOGRAPHY

Extracted proteoglycans in each group were combined and triplicate samples (500 μl) were applied to a Sephacryl S-500 column (1×30 cm) equilibrated with extraction buffer. The column was eluted with extraction buffer (20 ml/h) and fractions were analysed for proteoglycans.

ASSOCIATIVE SEPHACRYL S-1000 GEL FILTRATION CHROMATOGRAPHY

Aliquots of the pools of proteoglycans (100 μg glycosaminoglycan) were transferred into 0·05 M Na$_2$PO$_3$, pH 7·0, including 0·15 M NaCl, 0·5% CHAPS, 10 mM Na$_2$SO$_4$ and protease inhibitors by a PD-10 column, and incubated with 2% hyaluronan (w/w of total uronic acid, Healon, Pharmacia) overnight at 4°C. Triplicate Sephacryl S-1000 chromatography (1 cm×30 cm column) was performed for each sample to estimate the proportion of proteoglycans able to reaggregate with hyaluronan. The flow rate was 20 ml/hour and fractions (500 μl) were analysed for proteoglycans and radioactivity.

RELATIVE GLYCOSAMINOGLYCAN CHAIN LENGTHS

The relative chain lengths of glycosaminoglycans remaining after keratanase and chondroitinase ABC treatments were compared by Sephacryl S-300 gel chromatography. Residual and GuCl extracted glycosaminoglycans were

studied separately. The extracts and proteinase K digested residues were desalted, lyophilised, and reduced by borohydride as described earlier. Specific enzyme digestions were then performed after another desalting and lyophilisation. A Sephacryl S-300 gel column (1×30 cm) was eluted with 0·1 M Na-phosphate, pH 7·0, 0·5% CHAPS, 0·15 M NaCl, and 10 mM Na_2SO_4.

CHONDROITIN SULPHATE DISACCHARIDE ISOMERS

Extracted proteoglycan samples (10 μg of uronic acid) were digested with chondroitinase AC II (from *Arthrobacter aurescens*, EC 4.2.2.5; Seikagaku Co). Chondroitin sulphate isomers and hyaluronic acid were separated by thin layer chromatography[23] and molar proportions measured from scans obtained by a Shimadzu CS-930 chromatogram spectrophotometer (Kyoto, Japan) at 232 nm. The proportion of labelled chondroitin 6-sulphate and chondroitin 4-sulphate were estimated from Hyperfilm β-max film (Amersham Int) after a two week exposure. The digitised image of the film was analysed by Image software.

GLUCOSAMINE: GALACTOSAMINE RATIO

The rest of the GuCl extracted sample pools were hydrolysed in 2 M HCl for 17 hours at 103°C to produce an optimal yield of hexosamines from glycosaminoglycan chains.[24] The acid was evaporated by air flow and internal standard (deoxyglucose) and water was added, dissolving the hydrolysate into 2 ml. The hydrolysates were analysed on a Carbopak PA1 column by a Dionex HPLC system equipped with a pulsed amperometric detector and computer interface (Dionex, Sunnyvale, CA). The monosaccharides were separated by isocratic elution with 16 mM NaOH (flow 1·0 ml/minute, run time 20 minutes).

STATISTICAL ANALYSIS

The statistical significance between the runner and control groups was analysed by Wilcoxon's matched pairs signed ranks test.

Results

No difference in the body weights was found between control (mean 9·9 (SD 1·0) kg) and experimental groups (9·7 (1·1) kg), although food consumption of the runners was 50% higher than that of controls. The running itself

was considered aerobic as the pulse rate of the runners did not exceed 130 beats/min.[25]

The articular surface of the femoral head was checked for the quality and possible damage caused by the running exercise. No macroscopic injury, osteophytes, or fibrillation could be seen in any of the animals. Concentration of DNA varied a lot in individual specimens (table 1), as noted earlier,[26] but the mean values were similar in every group.

The rate of sulphate incorporation (pmol/mg wet weight/hour) was a little higher in the weight bearing area (fig 2), but only slight differences (not statistically significant) were noticed between groups. The rates were in good agreement with those obtained earlier in canine knee joints.[8] Uronic acid concentrations were similar to those reported previously.[27] Statistically significant changes were found in the posterior area of the femoral head (less weight bearing, table 1). A loss of extractable proteoglycans in the runner group (p≤0·02) was compensated for by an increase in proteoglycans resistant to extraction (p≤0·05). A statistically significant decrease in the extraction percentage was noted in the less weight bearing area also (p≤0·05). Running apparently raised non-extractable proteoglycans in the less weight bearing area close to that of the weight bearing area.

Quantitative autoradiography was used to obtain the distribution profile of newly
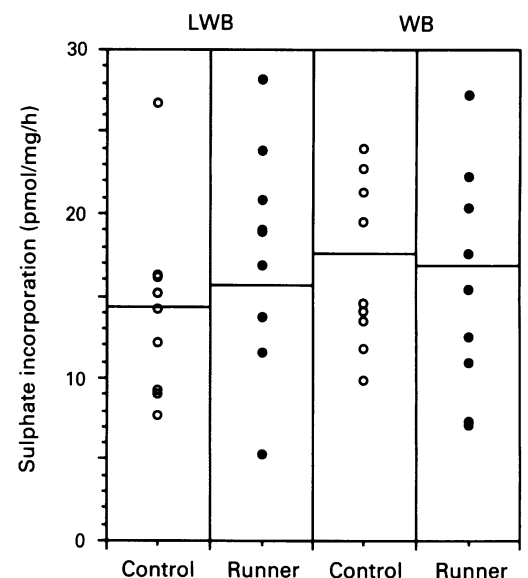
Figure 2 Sulphate incorporation rates from individual dogs (nine in each group) after one year of running exercise. Posterior (less weight bearing (LWB)) and anterior (weight bearing (WB)) areas were studied separately and the results were corrected for tissue wet weight. The mean of the rates in each group is shown by a horizontal line.

Table 1   Mean (SD) of the chemical measurements and incorporation rates in the low weight bearing (LWB) and high weight bearing (WB) areas of the canine femoral head (n=8–10)

	LWB control	WB control	LWB runner	WB runner
μg DNA/mg wet weight	0·49 (0·24)	0·50 (0·21)	0·48 (0·28)	0·49 (0·26)
Incorporation rate (pmol SO_4/mg/h)	14·1 (5·7)	17·6 (6·8)	15·8 (6·5)	16·8 (5·2)
Uronic acid/mg wet weight (extractable)	10·1 (2·8)	7·3 (3·1)	9·4 (2·7)**	9·0 (1·9)
Uronic acid/mg wet weight (residual)	2·0 (0·4)	2·4 (1·3)	2·7 (0·6)*	2·6 (1·4)
Extractability (%)	83·1 (3·9)	75·4 (4·2)	78·5 (4·2)*	77·7 (6·5)

*p≤0·05, **p≤0·02, Wilcoxon's matched pairs signed ranks test (control and runner groups from each area were tested separately).
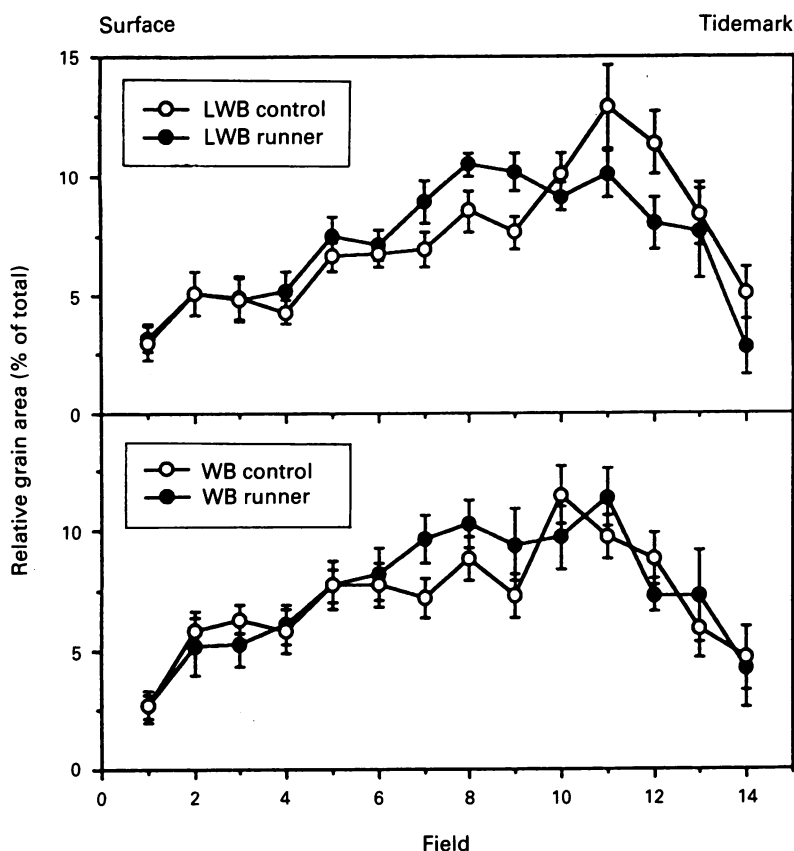
Figure 3   Distribution of sulphate incorporation in the posterior (less weight bearing (LWB)) and anterior (weight bearing (WB)) areas analysed by quantitative autoradiography. Relative grain areas in consecutive fields extending from cartilage surface to tidemark were measured by image analysis and the percentage distribution profiles were plotted.

synthesised proteoglycans in the tissue. Successive fields from surface to tidemark were analysed and the results were normalised to show the labelling density of each field in relation to the sum of all fields from surface to tidemark. As seen from the profiles (fig 3) the highest labelling density was noticed in the deep zone of the cartilage. After long distance running the synthetic activity in the intermediate zone was slightly increased in both less weight bearing and weight bearing areas, although the change did not reach statistical significance.

Specific enzymatic digestions were used to estimate the percentage of sulphate incorporated into keratan sulphate of the proteoglycans. Table 2 shows that a decrease in the relative amount of newly synthesised keratan sulphate to chondroitin or dermatan sulphate was detected by Sephacryl S-300 gel chromatography as a consequence of running exercise. The ratio of glucosamine to galactosamine in the extracted proteoglycans was similar in the weight bearing and less weight bearing areas of control animals. A decrease in glucosamine

(representing keratan sulphate) was found in the runner group (table 2). The results thus suggest a reduced synthesis and content of keratan sulphate (relative to chondroitin sulphate) after long term running.

The size distribution of proteoglycan monomers of individual specimens was examined by SDS agarose gel electrophoresis and from pooled samples by Sephacryl S-500 gel chromatography under dissociative conditions. Toluidine blue stained agarose gel showed two slowly migrating bands consisting of the large proteoglycans and a higher mobility band of small proteoglycans at R_f 0·8 in all groups (fig 4). Table 3 presents the percentage proportions of the large and small proteoglycans determined from toluidine blue stained gels. A different pattern was produced by autoradiography of the same gels (fig 4). Most of the newly synthesised proteoglycans were in the slower migrating band of the large proteoglycans (fig 4), and a much higher proportion of the radioactive precursor was incorporated into the small proteoglycans, as would be expected on their amount present in the tissue, suggesting a relatively higher turnover rate for small proteoglycans. Running slightly diminished the percentage of radioactive labelling of the small proteoglycans (NS).

Sephacryl S-500 gel chromatography of samples gave roughly similar profiles for both groups. As a whole, the results were in good agreement with those acquired from agarose gel electrophoresis. The differences were small and no significant increase in the size of proteoglycans of the runners could be noticed.

The proportion of proteoglycans able to form aggregates was studied by Sephacryl S-1000 gel chromatography with excess hyaluronan added to proteoglycan samples. Samples pooled separately for each group gave similar results in triplicate runs (fig 5). Some 50–60% of the tissue proteoglycans were able to aggregate, whereas only about 25% of the newly synthesised did aggregate. The groups did not differ from each other in their aggregation properties.

The relative chain lengths of glycosaminoglycans were compared by Sephacryl S-300 gel chromatography of keratanase and chondroitinase ABC digests (fig 6). Safranin O precipitation was used to quantify the distribution of resident proteoglycans and the radiolabel of both the GuCl extracted and residual glycosaminoglycans. In the weight bearing pool, extracted proteoglycans of the runners had somewhat larger chondroitin sulphate chains than controls whereas in less weight bearing extracts the elution of chondroitin sulphate of the runner group

Table 2   Percentage proportions of sulphate incorporation into chondroitin and dermatan sulphate and keratan sulphate estimated by Sephacryl S-300 gel chromatography after enzymatic digestions by keratanase and molar ratio of glucosamine to galactosamine in extracted proteoglycans

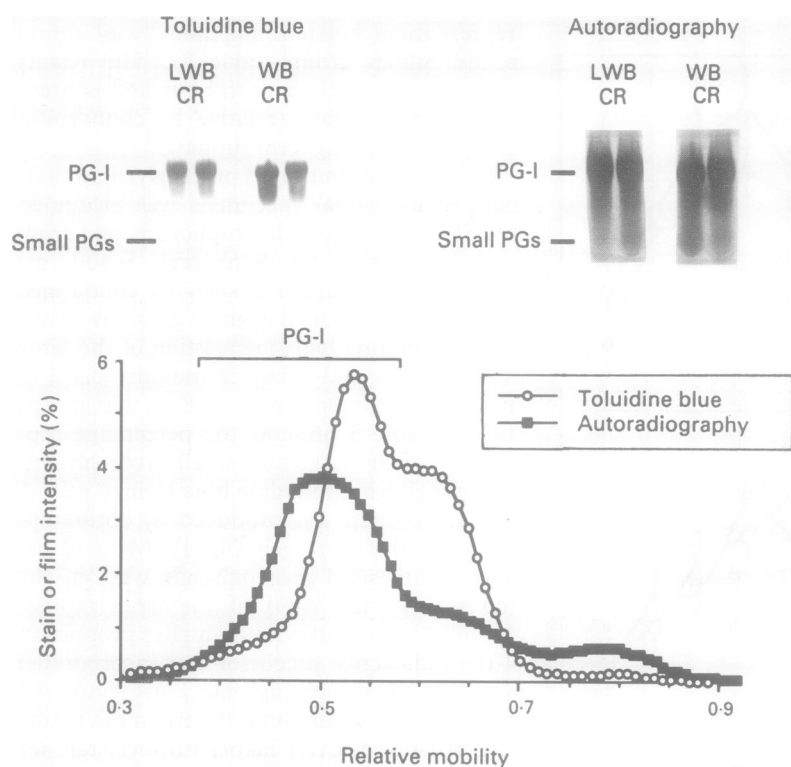|	LWB control	WB control	LWB runner	WB runner
Chondroitin+dermatan sulphate (%)	74·1	80·6	76·6	82·2
Keratan sulphate/chondroitin sulphate ratio	0·35	0·24	0·30	0·22
Glucosamine/galactosamine ratio	0·28	0·29	0·26	0·24

LWB=less weight bearing; WB=weight bearing.

Figure 4 Proteoglycans analysed by sodium dodecyl sulphate (SDS) agarose gel electrophoresis. Gels were stained with toluidine blue and an autoradiography film was exposed to the radioactivity of the gel for two weeks. A representative densitometric graph is shown for toluidine blue and autoradiography from the same sample lane. The area covered by slowly migrating large proteoglycans (PG-Is) is presented in the densitogram, and its migration position is shown in the gel. The position of the small proteoglycans (small PGs) is also shown (C=control and R=runner animal, LWB=posterior, less weight bearing area and WB=anterior, weight bearing area).

Table 3 The results of SDS agarose gel electrophoresis of proteoglycans

	LWB control	WB control	LWB runner	WB runner
Toluidine blue staining:				
Proteoglycan proportions:				
Large proteoglycans	98·8 (0·6)	98·8 (0·6)	98·8 (0·2)	98·7 (0·9)
Small proteoglycans	1·2 (0·6)	1·2 (0·6)	1·2 (0·2)	1·3 (0·9)
Large proteoglycans				
% PG-Is	66·2 (6·6)	68·2 (5·9)	64·8 (5·7)	70·3 (5·8)
Autoradiography:				
Proteoglycan proportions:				
Large proteoglycans	91·9 (2·8)	92·1 (1·8)	92·7 (4·2)	93·6 (2·4)
Small proteoglycans	8·1 (2·8)	7·9 (1·8)	7·3 (4·2)	6·4 (2·4)
Large proteoglycans:				
% PG-Is	73·1 (7·3)	74·0 (7·7)	75·1 (8·0)	75·7 (7·0)

In each group, all samples (n=10) were run in agarose gels, stained with toluidine blue and autoradiography films were exposed to the gels. The definition of PG-I is explained in fig 4.

remained much the same as the control. Keratan sulphate chains were heterogeneous in control animals, but changed into a more homogeneous population after the running exercise. Residual glycosaminoglycans of both groups were similar in their elution profiles.

The proportions of chondroitin sulphate isomers and hyaluronan disaccharides were examined by thin layer chromatography.[23] No significant alterations were noticed in any of the disaccharides (table 4). The percentage of hyaluronan in the residual glycosaminoglycans in control and runner groups was about twice as high as that found in GuCl extracted proteoglycans.[13] This supports our own findings that a large proportion of the hyaluronan remains in the tissue after GuCl extraction of 20 μm thick sections (unpublished data). The ratio of chondroitin 6-sulphate to chondroitin 4-sulphate was higher in the newly synthesised

proteoglycans, especially in the less weight bearing samples (table 4), but the change was not statistically significant. The values obtained for the hyaluronan percentage of total glycosaminoglycans were in good agreement with an earlier estimate from canine knee joints.[28]

Discussion

The adaptation of the femoral head articular cartilage of young beagles to long distance running was remarkably good as only minor biochemical changes were found in the structure and metabolism of the proteoglycans of the extracellular matrix after one year of training with the final daily running distance of 40 km with 15° uphill inclination. These findings are by contrast with an earlier report by Vasan that showed fibrillation and wear of the canine femoral head after six to eight miles (9·6–12·8 km) of daily running for eight months (one hour a day) with 20° uphill inclination.[3] A different running programme (faster speed and steeper inclination of the treadmill) possibly explains the severe degenerative alterations produced in his experiment.[3] The breed and especially the age of the dogs (not reported by Vasan) may also significantly affect the response of cartilage to exercise.

The chemical composition and tensile properties vary according to depth[29–31] and topographical site of the articular cartilage.[26][32–34] On the other hand, concentration of collagen seems to be relatively constant at different depths of the cartilage.[35] The tensile stiffness of cartilage correlates well with the hydroxyproline: hexosamine ratio.[36] Indentation tests have indicated that the highest compressive stiffness in the femoral head of humans resides in a region complementary to the loaded area of acetabulum,[37] whereas in quadruped animals weight bearing is focused more to the anterior part of the caput.[38]

Different responses in proteoglycan content after the running were apparent in the less weight bearing and weight bearing areas. Running increased the amount of GuCl resistant glycosaminoglycans particularly in the less weight bearing tissue. A similar increase of GuCl resistant glycosaminoglycans was reported in rabbits after both physical exercise and increased weight bearing,[39–41] whereas decreased weight bearing in a splinted leg clearly reduced non-extractable glycosaminoglycans.[41] This could indicate the presence of a stiffer collagen network after increased weight bearing spatially entrapping proteoglycans in the collagen network. Also, increased amounts of type IX collagen, which is covalently linked to collagen type II,[42] can be involved in the accumulation of residual glycosaminoglycans, as type IX collagen contains chondroitin or dermatan sulphate.[43] Conversely, in osteoarthritis the amount of non-extractable glycosaminoglycans was diminished.[7] An intact collagen network is crucial to the function of cartilage. When exposed to leucocyte elastase (attacking the non-helical
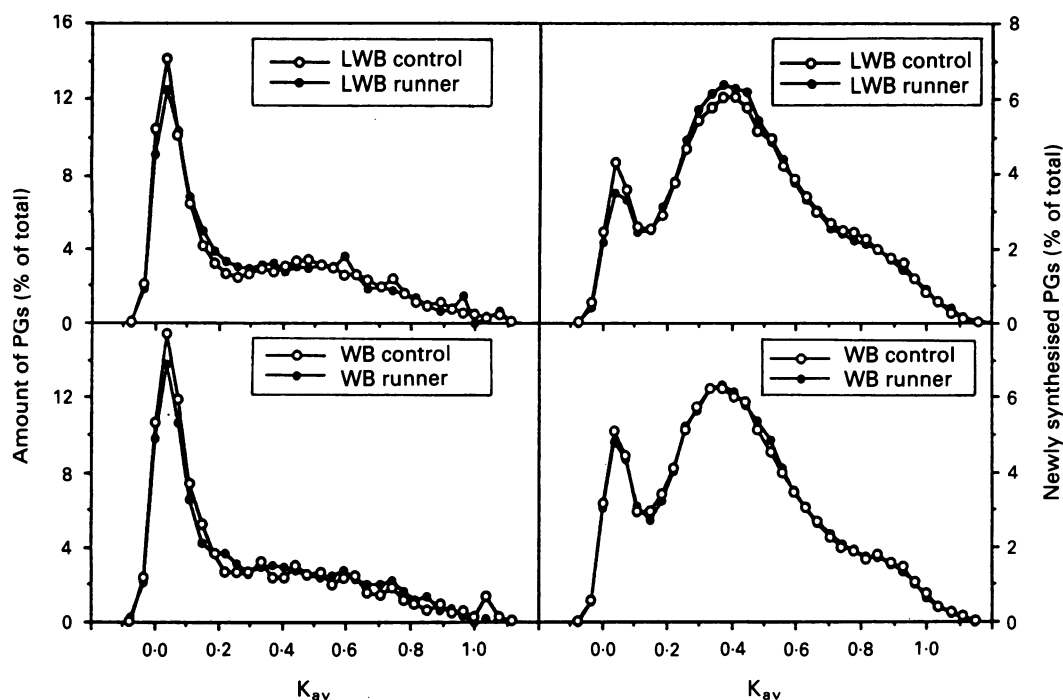
Figure 5   Aggregation capacity of extracted proteoglycans (PGs) analysed on Sephacryl S-1000 gel filtration column. Proteoglycans were allowed to form aggregates in the presence of exogenous hyaluronan (2% w/w of uronic acid) overnight at 4°C. Fractions were analysed for total and newly synthesised proteoglycans by the safranin O precipitation methods. LWB=less weight bearing; WB=weight bearing.
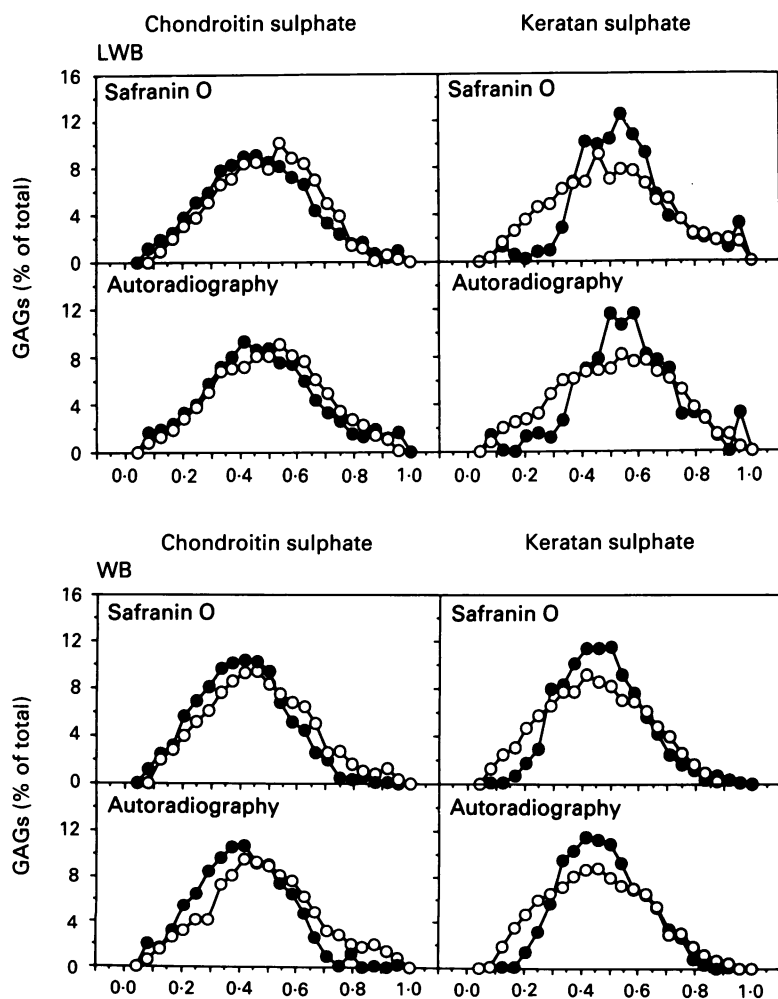

Figure 6   Glycosaminoglycan (GAG) chain size distribution of chondroitin or dermatan sulphate and keratan sulphate of the extracted proteoglycans. Proteoglycan samples were reduced by alkaline borohydride and digested separately by keratanase and chondroitinase ABC respectively before analysis on Sephacryl S-300. Glycosaminoglycans in the fractions were determined by the safranin O precipitation method. (O) represent control and (●) runner group. LWB=less weight bearing; WB=weight bearing.

terminal regions of type II and possibly type IX collagen) reduction of the elastic stiffness of cartilage was caused.[44]

In this study, the highest synthetic activity in control animals was in the deep layers of the cartilage revealed by quantitative autoradiography and an increase in the intermediate zone was noticed after running exercise. A similar statistically significant change in the distribution of proteoglycan synthesis has been found in young guinea pigs after 18 weeks of treadmill running exercise (Hyttinen et al, unpublished data). In our earlier in vitro experiments, cyclic mechanical compression promoted sulphate incorporation by cartilage explant in the area under loading, an increase localised mainly in the intermediate zone.[45]

The ratio of glucosamine to galactosamine is a good indicator of the proportion of keratan and chondroitin sulphates, even in crude extracts. A decreased ratio in the runner group suggested a relative increase of chondroitin sulphate, a trend similar to the results obtained in sheep by increased weight bearing.[46] A contradictory finding with increased keratan sulphate after enhanced loading was made in rabbits.[41] In Sephacryl S-300 chromatography slightly longer chondroitin sulphate chains were noticed in the runner group, which may well explain the trend towards an increase in the chondroitin:keratan sulphate ratio. In experimental canine osteoarthritis, proteoglycans containing more chondroitin sulphate relative to keratan sulphate appeared in the tissue.[7] Whether the change in keratan:chondroitin sulphate ratio, noticed in this experiment, reflects an increased stress and weight bearing due to the inclination of the running or an early osteoarthritic process remains an open question.

Table 4   Percentage proportions of chondroitin sulphate isomer and hyaluronan disaccharides produced by chondroitinase AC and separated by thin layer chromatography (TLC). The ratio of radioactively labelled sulphated disaccharides was obtained by an exposure of an autoradiography film to the TLC plate

	LWB control	WB control	LWB runner	WB runner
UV absorbing disaccharides:				
CS-6	61·6 (7·3)	62·8 (6·6)	63·0 (5·3)	60·6 (4·9)
CS-4	18·6 (2·2)	21·0 (3·8)	20·4 (2·9)	22·2 (2·5)
CS-0	8·7 (3·5)	8·5 (3·1)	7·5 (4·2)	9·2 (3·6)
HA	11·1 (4·6)	7·7 (3·8)	9·1 (2·9)	8·0 (2·4)
CS-6/CS-4	3·4 (0·6)	3·1 (0·7)	3·1 (0·5)	2·8 (0·4)
Radiolabelled disaccharides:				
CS-6/CS-4	4·4 (1·3)	3·4 (1·2)	4·6 (1·1)	3·8 (1·0)

There was no other evidence of osteoarthritic alterations due to the running programme. The lower extractability of proteoglycans and unchanged incorporation rates rather suggested a tendency opposite to degeneration.[3][8][10][47] We suggest that the enhanced load distributed the stress over a larger articular surface area and initiated an adaptation reaction particularly in the previously less weight bearing sites. The findings by Volpi and Katz[38] suggest that cartilage has the capacity to respond to various mechanicochemical histories, as the potential of superficial collagen fibrils to "hyperswell" was noticed to develop only in the anterior, weight bearing region of bovine femoral heads in response to different loading environments.

The general shape of the hip joint is congruent, which minimises the local impacts. With the same running programme, the more incongruent femoral condyles of the knee showed a depletion of proteoglycans in the superficial zone after long distance running (Arokoski *et al*, unpublished data). This emphasises the importance of a local response and the congruency of the joint surface in the maintenance of the normal cartilage. The results also suggest that congruency, by controlling the strength of local impacts, is an important factor in determining the response of cartilage to exercise.

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