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Key terms: ³⁵S-sulfate; animal experiment; articular cartilage; biochemical study; effect; glycosaminoglycan; motion load; motion load change; rabbit; scanning electron microscopic study; scanning electron microscopy; tendon; tendon tissue

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Effects of motion load changes on tendon tissues and articular cartilage

A biochemical and scanning electron microscopic study on rabbits

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VIDEMAN, T., ERONEN, I. and CANDOLIN, T. Effects of motion load changes on tendon tissues and articular cartilage: A biochemical and scanning electron microscopic study on rabbits. *Scand. j. work environ. & health* 5 (1979): suppl. 3, 56—67. The motion load of rabbits was increased by treadmill running during a 5-d period. One group ran on a level surface, while the other ran uphill. After the running, the glycosaminoglycan metabolism and scanning electron microscopic photographs of different tendon, ligament and cartilage samples were analyzed. Uphill running decreased the glycosaminoglycan metabolism of articular and costal cartilage. The clearest irregularities of articular cartilage studied by scanning electron microscopy were also seen in the uphill running group. Increasing the motion load increased the glycosaminoglycan synthesis of stretched tendons. The possible combination of general inhibitory and local stimulating effects is discussed.

Key words: animal experiments, articular cartilage, glycosaminoglycans, motion load, ³⁵S-sulfate, scanning electron microscopy, tendon.

The etiologic factors of occupational rheumatic diseases are not fully understood. The causative factors of work can be disturbances in blood circulation, neuromuscular dysfunction, altered connective tissue metabolism, or mechanical overstrain (17, 18). The exact effect of increasing tension or repetitive movements on the metabolism of glycosaminoglycan and collagen in tendon tissue and cartilage is not known. These effects of motion load have generally been studied in connection with physical training, growing or ageing,

and the results have primarily been based on physical and morphological parameters after relatively long-term experiments (11, 12, 13, 21, 22, 25).

Biochemical studies have shown such changes in connective tissue matrix proteins as an accelerated turnover of collagen and other proteins in tendons and ligaments and increased concentrations of hexosamines and nitrogen in skin after training (10). In addition enhanced activities of metabolic and other enzymes have been reported after physical exercise (14). In this study we have concentrated on comparing the effects of two different motion loads and determining the initial phenomena occurring after a many-fold increase in normal motion load. The rabbit, which normally moves little, is suitable for experiments with marked increases in motion load.

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Glycosaminoglycans (GAG) are macromolecules consisting of repeating disaccharide units, which can be sulfated (chondroitinsulfates, keratansulfate) or nonsulfated (hyaluronic acid). They occur in tissues as protein complexes, known as proteoglycans, and possess unique chemical properties. The special mechanical properties of cartilage result from the entrapment of proteoglycans within the collagen network of the cartilage, and the integrity of this structure is essential for optimum joint performance (8). A decreased concentration of GAG is assumed to alter the plasticity of soft connective tissue and to reduce lubrication efficiency (1, 5, 6).

Scanning electron microscopy (SEM) may be the most sensitive method for verifying initial changes in connective tissue surfaces, as it is in detecting possible fibrillation or microtraumatization. GAG metabolism was selected as an indicator of connective tissue matrix metabolism because of its relatively high turnover rate when compared with that of collagen and because of its effects on connective tissue biomechanics.

MATERIAL AND METHODS

Scanning electron microscopy

The material consisted of 15 adult rabbits. Three of the rabbits served as controls, and the remainder (12 rabbits) (table 1) were made to run on a treadmill (fig. 1) three times daily for 5-d at a speed of 0.2 m/s. The total running distance was 800 or 2,400 m in 5 d (= change in number of repetitive movements), and the inclination of the treadmill was either 0° or 20° (= change in forces affecting joint structures). The hind legs of three of these rabbits were always in an ischemic condition 2 h before the running. The ischemic condition was accomplished with elastic bands.

Samples for the SEM consisted of *fascia corresponding to the human extensor retinaculum, the tendon of the tibial anterior muscle, patella, and medial condyle of the tibia*. After the rabbits were

killed, the samples were fixed in a 2.5 % glutaraldehyde solution, dehydrated and gilded by vacuum steaming. Photographs were taken from the corresponding areas of the samples with magnifications of

Table 1. Running distances and degrees of treadmill inclination used for the exercising rabbits.

Rabbit number and group	Running distance (m) during 5 d	Degree of inclination
<i>Scanning electron microscopic experiments</i>		
42 } A	800	0
56 } A	800	0
73 } A	800	0
44 } B	800	0
58 } B	800	0
63 } B	800	0
43 } C	800	20
49 } C	800	20
64 } C	800	20
52 } D	2,400	0
57 } D	2,400	0
65 } D	2,400	0
<i>Biochemical experiments</i>		
120	3,700	0
122	2,880	0
123	6,440	0
135	6,530	0
137	7,580	0
143	7,210	0
128	8,060	20
129	6,225	20
130	5,765	20
131	3,680	20
139	7,100	20
140	7,050	20

Fig. 1. The treadmill used in this experiment.

Table 2. Total uronic acid concentration of the samples ($\mu\text{g}/\text{mg}$ dry, defatted tissue). Means and standard deviations are given.

Sample origin	Control group	Level-surface group (N = 6)	Uphill group (N = 5)
Tibial articular cartilage	33.6 $\pm$ 4.2 (N = 9)	34.4 $\pm$ 1.7	30.3 $\pm$ 2.9
Femoral articular cartilage	33.0 $\pm$ 6.9 (N = 9)	27.9 $\pm$ 3.5	30.3 $\pm$ 3.9
Medial meniscus	5.83 $\pm$ 0.99 (N = 9)	6.84 $\pm$ 1.23	7.05 $\pm$ 1.21*
Medial collateral ligament	1.81 $\pm$ 0.30 (N = 9)	1.62 $\pm$ 0.34	1.91 $\pm$ 0.55
Achilles tendon	0.73 $\pm$ 0.27 (N = 9)	0.64 $\pm$ 0.12	0.59 $\pm$ 0.08
Peritenon of the Achilles tendon	1.61 $\pm$ 0.18 (N = 9)	1.62 $\pm$ 0.21	1.47 $\pm$ 0.13
Extensor retinaculum	4.24 $\pm$ 0.20 (N = 3)	4.23 $\pm$ 0.65	3.81 $\pm$ 0.42
Tendon of tibial anterior muscle	1.65 $\pm$ 0.44 (N = 3)	1.60 $\pm$ 0.18	1.46 $\pm$ 0.21
Costal cartilage	43.6 $\pm$ 6.0 (N = 6)	44.8 $\pm$ 3.3	43.5 $\pm$ 0.4

* $p < 0.05$ (versus control group).

Table 3. ^{35}S -radioactivity of the samples (DPM/ μg uronic acid).

Sample origin	Controls (Mean $\pm$ SD)	Level-surface group (N = 6)		Uphill group (N = 5)	
		Mean $\pm$ SD	p	Mean $\pm$ SD	p
Tibial articular cartilage	93 $\pm$ 21 (N = 9)	98 $\pm$ 17		49 $\pm$ 14	< 0.025 (vs. controls)
Femoral articular cartilage	100 $\pm$ 29 (N = 9)	130 $\pm$ 28		58 $\pm$ 25	< 0.025 (vs. controls) < 0.005 (vs. level-surface group)
Medial meniscus	179 $\pm$ 124 (N = 9)	156 $\pm$ 53		77 $\pm$ 43	
Medial collateral ligament	162 $\pm$ 47 (N = 9)	108 $\pm$ 43	< 0.05 (vs. controls)	48 $\pm$ 28	< 0.005 (vs. controls)
Achilles tendon	120 $\pm$ 36 (N = 9)	190 $\pm$ 53	< 0.01 (vs. controls)	104 $\pm$ 41	< 0.005 (vs. level-surface group)
Peritenon of the Achilles tendon	148 $\pm$ 56 (N = 9)	176 $\pm$ 41		186 $\pm$ 50	
Extensor retinaculum	112 $\pm$ 20 (N = 3)	135 $\pm$ 17		116 $\pm$ 47	
Tendon of tibial anterior muscle	186 $\pm$ 32 (N = 3)	329 $\pm$ 75	< 0.05 (vs. controls)	227 $\pm$ 83	< 0.05 (vs. level-surface group)
Costal cartilage	14 $\pm$ 3 (N = 6)	12 $\pm$ 2		9 $\pm$ 1	< 0.005 (vs. level-surface group) < 0.005 (vs. controls)

Fig. 2. SEM photographs of the articular cartilage of the medial tibial condyle. The upper picture represents the level-surface running group and the lower one the uphill running group. The running distances were the same for both groups. Clear degenerative changes can be seen on the articular surface in the lower picture (magnification 500 $\times$).

50, 150, 500, and 1,500 $\times$. Two persons assessed the photographs independently using the following subjective scale: 0 = no degenerative changes, 1 = slight, 2 = moderate, and 3 = severe degenerative changes [corresponding to the classification of Kotani et al. (16) in the following way: 0 = 1, 1 = 2, 2 = 4 and 3 = 6]. They paid special attention to the fibers and "scaly" irregularities of the surfaces.

Three rabbits, living in their coops, served as a control group, and one leg of each rabbit was used for the SEM study. An arthrotic control group, produced by immobilization, was also used. The methods used with and the results obtained from this group have been described earlier (24).

The results of the SEM study were based on 306 photographs, but they were not

Fig. 3. Degenerative changes on the surface of articular cartilage of the medial tibial condyles. The changes of the uphill running group (C) are similar to those of the arthrotic group (produced by immobilization), and the changes in the other running group resemble those of the control group (rabbits living in their coops).

analyzed statistically because the groups consisted of only three rabbits each.

Biochemical methods

The GAG analysis was made with 12 adult rabbits which ran three times daily up to exhaustion throughout a 5-d period (table 1) and 9 control animals living in their coops. The running distances and inclinations are listed in table 1. The running speed was 0.30–0.50 m/s. (One rabbit made to run uphill was excluded because of abnormally high levels of GAG and radiolabeled incorporation in all its analyzed tissues.)

On the fifth day, after the last training exercise, the animals were injected with ^{35}S -sulfate (carrier-free, Amersham, 0.3 mCi/kg) and killed 24 h later. Samples were taken from both hind legs with a scalpel. They consisted of *tibial and distal femoral articular cartilage of the knees, costal cartilage, medial meniscus, medial collateral ligament of the knees, Achilles tendon, peritenon of the Achilles tendon, fascia corresponding to the human extensor retinaculum, and tendon of the tibial anterior muscle.*

The procedures used for the sample handling and the separation of GAGs from the tissues have been published elsewhere (7). The analyses of the separated GAGs consisted of determining the uronic acid (UA) (3), galactosamine (Gala) and glucosamine (GluA) (19) contents of articular

cartilage and measuring the total ^{35}S -radioactivity with liquid scintillation.

The radioactivity was counted with a LKB-Wallac 81000 liquid scintillation counter according to an external standard channels ratio method for DPM (disintegrations per minute) calculation. The scintillation system consisted of xylene, triton X-100 and dibutylphosphate (75:25:1) containing 2,5-diphenyl oxazole (PPO, 5 g/l) and p-bis(o-methylstyryl) benzene(bis-MSB, 0.5 g/l). The GAGs of the Achilles tendon and corresponding peritenon were fractionated with DEAE-cellulose (4) in micro columns (0.7×2.5 cm) to hyaluronic acid (HA) and sulfated glycosaminoglycans (S-GAG), which were then quantified by UA analysis. The concentrations were calculated as micrograms per milligram of dry, defatted tissue, and the specific radioactivities as DPM per microgram of UA after the decay of radioactivity had been corrected to the reference date of the isotope.

The results were evaluated statistically with a one-tailed Mann-Whitney nonparametric test. The differences were considered significant when p was < 0.05 .

RESULTS

In both the *tibial and femoral articular cartilage*, and in the *costal cartilage* as well, there was no significant difference in the UA concentration between the running and control groups (table 2).

Fig. 4. SEM photographs of the articular cartilage of the patella. The upper picture represents the level-surface running group and the lower one the uphill running group. There are more irregularities in the upper picture (magnification 500 $\times$).

Running on the uninclined treadmill, i.e., on a level surface, did not affect radio-sulfate incorporation in comparison with that of the controls, but running "uphill" decreased incorporation in all samples in a corresponding comparison with controls ($p < 0.025$) and in the femoral cartilage when compared to level-surface running ($p < 0.005$) (table 3). The SEM photographs from the surface of the articular cartilage of the tibia showed more irregularities in

the uphill group than in the control or level-surface group, but the postischemic level-surface group did not differ from normal (fig. 2 and 3). Macroscopically, no changes could be seen in the articular cartilage. Neither did an increase in motion load change the SEM picture of the articular surface of the patella (fig. 4 and 5). The GluA concentration was not affected in articular cartilage during motion load, while the GalA concentration

Fig. 5. Degenerative changes on the surface of the articular cartilage of the patella. The changes of the different running groups (A-C) are similar to those of the control group and less than those in the arthrotic group (produced by immobilization).

Table 4. Analysis of galactosamine (GalA) and glucosamine (GluA) content of articular cartilage.

	Controls (N = 9) (Mean $\pm$ SD)	Level-surface group (N = 6)		Uphill group (N = 5)	
		Mean $\pm$ SD	p	Mean $\pm$ SD	p
<i>Tibial articular cartilage</i>					
GalA (μ g/mg)	28.2 $\pm$ 6.2	25.3 $\pm$ 2.6		24.5 $\pm$ 2.2	
GluA (μ g/mg)	11.1 $\pm$ 4.6	10.5 $\pm$ 2.5		12.1 $\pm$ 1.5	
GalA/uronic acid (molar ratios)	0.95 $\pm$ 0.21	0.99 $\pm$ 0.02		0.89 $\pm$ 0.10	< 0.05 (vs. level- surface group)
GluA/GalA (molar ratios)	0.41 $\pm$ 0.17	0.42 $\pm$ 0.08		0.50 $\pm$ 0.08	< 0.05 (vs. level- surface group)
<i>Femoral articular cartilage</i>					
GalA/mg	27.8 $\pm$ 5.1	33.8 $\pm$ 2.8	< 0.01 (vs. controls)	32.8 $\pm$ 3.9	
GluA/mg	19.7 $\pm$ 4.0	20.7 $\pm$ 3.7		20.7 $\pm$ 2.9	
GalA/uronic acid (molar ratios)	0.93 $\pm$ 0.18	1.07 $\pm$ 0.11	< 0.025 (vs. controls)	1.18 $\pm$ 0.09	< 0.0025 (vs. controls) < 0.025 (vs. level- surface group)
GluA/GalA (molar ratios)	0.74 $\pm$ 0.25	0.62 $\pm$ 0.11		0.64 $\pm$ 0.08	

was elevated in tibial and depressed in femoral articular cartilage in both running groups. The molar ratios of GalA/UA were

increased above the level of the controls in the tibial articular cartilage of both running groups ($p < 0.025$) and remained at

Table 5. Fractionation of glycosaminoglycans from Achilles tendon tissues by DEAE-cellulose.

Glycosaminoglycan fraction ^a	Controls (N = 7) (Mean $\pm$ SD)	Level-surface group (N = 6)		Uphill group (N = 5)	
		Mean $\pm$ SD	p	Mean $\pm$ SD	p
<i>Achilles tendon</i>					
HA (μ g/mg) ^b	0.33 $\pm$ 0.09	0.40 $\pm$ 0.11		0.35 $\pm$ 0.04	
S-GAG (μ g/mg) ^b	0.29 $\pm$ 0.08	0.28 $\pm$ 0.05		0.26 $\pm$ 0.06	
S-GAG/HA	0.91 $\pm$ 0.18	0.73 $\pm$ 0.19	< 0.01 (vs. controls)	0.76 $\pm$ 0.23	< 0.01 (vs. controls)
DPM/ μ g S-GAG	231 $\pm$ 92	437 $\pm$ 115	< 0.005 (vs. controls) < 0.005 (vs. uphill group)	230 $\pm$ 63	
<i>Peritenon of the Achilles tendon</i>					
HA (μ g/mg) ^b	1.12 $\pm$ 0.10	1.18 $\pm$ 0.30		1.06 $\pm$ 0.17	
S-GAG (μ g/mg) ^b	0.42 $\pm$ 0.04	0.45 $\pm$ 0.05		0.46 $\pm$ 0.04	
S-GAG/HA	0.38 $\pm$ 0.06	0.39 $\pm$ 0.07		0.44 $\pm$ 0.11	
DPM/ μ g S-GAG	492 $\pm$ 209	637 $\pm$ 143		563 $\pm$ 188	

^a HA = hyaluronic acid; S-GAG = sulfated glycosaminoglycans; DPM = radioactivity.

^b Measured as uronic acid per milligram of dry, defatted tissue.

a normal level in the corresponding femoral cartilage of both groups. The GluA/GalA ratio was increased in the femoral cartilage of the uphill running group in a comparison with the level-surface group ($p < 0.05$) (table 4).

For the *medial meniscus* no statistically significant differences between the different groups could be found. However, the GAG concentration of the uphill group rose to that of the control group ($p < 0.05$). On the other hand, the uphill group again showed lower specific radioactivity than either the level-surface or control group. In the *medial collateral ligaments* the GAG concentration was also similar in all the groups. Isotope incorporation was lower in both the running groups when compared to that of the controls (uphill vs. control group $p < 0.005$; level-surface vs. control group $p < 0.05$), but no significant difference occurred between the two running groups.

In the *Achilles tendons* no significant changes in GAG concentration could be found after the running. Radiosulfate incorporation in the level-surface group was higher than that of the control ($p < 0.01$) or uphill ($p < 0.005$) group. After fractionation of the GAGs of both running

groups the ratio of S-GAG to HA was decreased to a level lower than that of the controls (uphill vs. control group $p < 0.005$; level-surface vs. control group $p < 0.01$) (table 5). Radiosulfate incorporation into the S-GAG of the level-surface group was raised above the levels of the other groups (vs. control $p < 0.005$; vs. uphill group $p < 0.005$) (table 5).

In the *tendon of the anterior tibial muscle*, the GAG concentrations did not significantly differ between the groups. Isotope incorporation in the level-surface group was elevated above that of the other groups (vs. controls (3 animals) $p < 0.05$; vs. uphill group $p < 0.05$).

In the *retinaculum* no marked differences between the groups could be found (3 control animals and samples taken from one leg only). No signs of inflammation were seen in the tendon tissues.

No clear classification could be made between the SEM photographs of the different tendon tissues because the normal variation for SEM photographs from tendon surfaces is partly large and unknown (fig. 6 and 7).

In the *peritenon of the Achilles tendon* no significant changes between the dif-

Fig. 6. SEM photograph of the surface of the tendon of the tibial anterior muscle. The pictures are from different regions (magnification 1,500 $\times$).

ferent groups could be found before or after the fractionation of GAG.

The results can be summarized as follows: Increasing motion load changed the concentrations and ratios of GAG constituents mildly in articular cartilage and decreased radiosulfate uptake of GAGs in the articular and costal cartilage of the uphill group. Similar changes were also seen in the meniscus. Running on a level

surface accelerated the GAG metabolism of the tendons in the ankle region, and the uphill running had a similar, but not so clear, effect. The GAG metabolism of the collateral ligament decreased with increasing motion load. Total concentrations of the GAG were not affected in short-term training, but the ratio of S-GAG to HA may decrease in tendon tissues after motion load is increased.

Fig. 7. SEM photographs from the ligament corresponding to the human extensor retinaculum. The upper two pictures are from the level-surface running group, and the lower one is from the uphill running group. The irregularities are greatest in the uphill group, but the variation is also large in the other group (magnification 1,500 $\times$).

DISCUSSION

The purpose of this experiment was to study the effect of repetitive stretching and movements on GAG metabolism and surface structures of cartilage and tendons. We have no *exact* knowledge of the forces affecting single tissues of rabbits running

on different inclinations. However, we know from anatomic viewpoints that motion load is concentrated in the very small area in the articular cartilage of the tibia and that the pushing forces must be transmitted via the same area. In the distal femoral cartilage the motion load is distributed over a greater area. It is

also certain that running usually causes tension and/or friction in tendon tissues, but the mechanical strain of the collateral ligament is not so clear (21).

In our uphill running group a general inhibition of ^{35}S -sulfate incorporation into the GAGs was seen (also found in costal cartilage). In tendons physical stress evidently causes local stimulation, which increases synthesis and thus radioactivity to a normal level. The nature of inhibition is unclear, but one possible reason for the decreased incorporation of ^{35}S -sulfate into sulfated GAGs could be glucocorticoids, the levels of which can be increased during physical stress. The secretion of the adrenal cortex of rabbits, which normally consists predominantly of corticosterone, can, by prolonged ACTH (adrenocorticotropin) stimulation, (e.g., chronic stress) be shifted predominantly to cortisone (9). Glucocorticoids are known to inhibit protein synthesis (2) and thus the synthesis of sulfated glycosaminoglycans (15). In our level-surface group no such inhibition in radio-sulfate incorporation could be found; there was only a local stimulation in the tendons. These results with rabbits are understood best in respect to the general inhibiting effect and the local stimulating effect on GAG metabolism during running. The local stimulating effect is strong in stretched tendons, but in uphill running the general inhibiting effect can lower GAG synthesis.

Surface characteristics (in SEM) of articular cartilage in the medial tibial condyles of our uphill running group resembled those found under conditions of arthrosis. The alterations seen in the SEM photographs occurred in the thin surface zones of the cartilage, and GAG analyses dealing with sample thickness do not on the whole detect changes in these areas. The conclusions based on the SEM experiments of our study cannot be generalized with respect to the effect of running on articular cartilage because the nature of the changes was unclear and the increase in motion load abnormally large. The surface characteristics of the cartilage of our running groups appeared to be normal, and the ischemic conditions before running did not affect the SEM results. The running did not affect osteoarthritic changes in GAG metabolism (7, 23).

Before the changes observed in articular cartilage and tendons can be verified as symptoms of oncoming arthrosis and tendinitis, a series of experiments with an even greater and more prolonged motion load must be carried out.

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