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Cells and nuclei of articular cartilage chondrocytes in young rabbits enlarged after non-strenuous physical exercise*

KARI PAUKKONEN, KALEVI SELKÄINAHO†,
JUKKA JURVELIN, ILKKA KIVIRANTA
AND HEIKKI J. HELMINEN

Departments of Anatomy and †Applied Mathematics, University of Kuopio, Finland

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INTRODUCTION

The effects of altered joint loading and movement on articular cartilage have been studied using unloading (Olah & Kostenszky, 1972; Palmoski, Colyer & Brandt, 1980), excessive weight-bearing (Kostenszky & Olah, 1972; Caterson & Lowther, 1978; Tammi *et al.* 1983), compression with joint motion (Gritzka, Fry, Cheesman & LaVigne, 1973), peak overloading (Dekel & Weissman, 1978), and physical exercise models (Lanier, 1946; Ingelmark & Ekholm, 1948; Sääf, 1950; Krause, 1969; Dekel & Weissman, 1978; Videman, Eronen & Candolin, 1979; Kincaid & Van Sickle, 1982; Tammi *et al.* 1983; Jurvelin *et al.* 1985). Lack of loading (joint motion in the absence of normal loading) has been shown to cause degenerative articular changes, for example, decrease in cartilage thickness, loss of Safranin O staining of the matrix and glycosaminoglycan content, and an increase in water content of the tissue (Olah & Kostenszky, 1972; Palmoski *et al.* 1980). Excessive weight-bearing apparently results in acceleration of the ageing process in articular cartilage (Kostenszky & Olah, 1972; Tammi *et al.* 1983) or produces effects comparable to those observed in the early stages of osteoarthrosis (Caterson & Lowther, 1978). Compression with joint motion (Gritzka *et al.* 1973) and peak overloading (Dekel & Weissman, 1978) have been shown to lead to osteoarthrotic lesions.

The influence of physical exercise (running or other activities) on articular cartilage has not been investigated extensively. Using scanning electron microscopy, Videman *et al.* (1979) found minimal irregularities in the articular surface of rabbits running uphill but not for those running on a level surface; Stofft & Graf (1983) observed leaf-like surface defects on the articular cartilage of guinea-pigs; in the early stages of a running experiment, Jurvelin *et al.* (1985) detected transient striation of the articular surface of the rabbit knee. According to enzyme histochemical tests, there are no differences between the cartilage of physically exercised dogs and that of controls (Kincaid & Van Sickle, 1982). Running did not induce osteoarthrotic changes in glycosaminoglycan metabolism (Videman *et al.* 1979). In rabbits, running produces an increase in the proteoglycan content of articular cartilage matrix (Tammi *et al.* 1983).

* Reprint requests to Dr Kari Paukkonen, Department of Anatomy, University of Kuopio, P.O.B. 6, SF-70211 Kuopio, Finland.

In histological sections of the articular cartilage in strenuously exercised mice, Krause (1969) observed decreased stainability with Alcian blue and methylene blue but detected no structural changes. Lanier (1946) found that after one year of daily running, the runners had fewer minor histological lesions than did control mice. Sääf (1950) quantified histological changes in the humeroscapular joint of running adult guinea-pigs and observed that exercise increased the size of the chondrocytes.

The aim of this study is to examine the effects of non-strenuous physical exercise on articular cartilage in the knee joints of young rabbits. For this purpose, a new quantitative stereological method has been used (Paukkonen, Selkäinaho, Jurvelin & Helminen, 1984), which permits the detection even of small histological changes in the tissue structure of articular cartilage.

MATERIALS AND METHODS

Articular cartilage from 16 male New Zealand White rabbits (4·5–5 months old and weighing 2·8–3·7 kg) was examined. At the beginning of the experiment these animals were divided at random into control and experimental (running) groups. A horizontal treadmill was used to mobilise the running animals; the daily (five times a week) running distance increased from 300 m to 600 m (1st week, 300 m; 2nd week, 400 m; 3rd week, 450 m; 4th week, 500 m; 5th to 8th week, 600 m). The control rabbits lived in coops with a standard bottom area of 50 cm × 60 cm. Animals were anaesthetised with barbiturate (Nembutal®, Abbott, France) and then killed by air embolus. Using a Leitz 1600 saw microtome (Ernst Leitz Wetzlar GmbH, Wetzlar, FRG) the lateral tibial condyle of the right knee was sawn into slices perpendicular to the articular surface; under a stereomicroscope, cartilage blocks (30–40 per condyle) with surface dimensions of 0·3 × 1·5 mm were dissected free from the subchondral bone. Tissue blocks from the outermost rim of the condyle (bone–cartilage junction) were rejected. Blocks were fixed in 2% purified glutaraldehyde buffered to pH 7·3 with 0·15 M cacodylate buffer, partially decalcified (overnight) in 4% EDTA, dehydrated, and embedded in Epon according to the method of Shepard & Mitchell (1977). Using simple random sampling, six blocks were chosen for analysis. One section 1 μm thick was cut perpendicular to the articular surface and stained with iron haematoxylin and Safranin O (Sevier & Munger, 1968; Warmke & Lee, 1976) for light microscopical examination with a Wild M 501 semiautomatic microscope (Wild Heerbrugg Ltd, Heerbrugg, Switzerland).

Using a measuring ruler and a magnification of ×280, the thickness of the articular cartilage was measured from the articular surface to the tidemark (the lower border of the uncalcified cartilage). The mean cartilage thickness of the population, P_0, was:

$$P_0 = 1/q \sum_{k=1}^{q} 1/n \sum_{i=1}^{n} P_{ki}, \quad (1)$$

where i equals a section ($i = 1, 2, \ldots n$); k equals an animal ($k = 1, 2, \ldots q$).
As estimators of stereological ratios, volume density (V_V) and numerical density (N_V) of cells were determined by point counting at a magnification of ×1400, using a simple square lattice 9·8 × 9·8 cm with 49 points as a test system (the test system was fixed on the projection head of the Wild M 501 microscope). Data were collected separately from the superficial, middle and deep zones of the articular cartilage

(Meachim & Stockwell, 1979; Paukkonen *et al.* 1984). The zone boundaries were defined as follows: superficial zone: thickness constant, 5·6 cm at a magnification of ×1400 which included the layer of discoidal, flattened cells; the thickness of the middle zone was equal to $(P_{kt}-1{\cdot}12)/2$ where $1{\cdot}12 = 5{\cdot}6\times280/1400$; the thickness of the deep zone was the same as that of the middle zone. The size frequency distribution and mean diameter of the nuclei ($\bar{D}$) were estimated according to the method described by Weibel (1979) using a semiautomatic image analyzer (Summagraphics ID-2-CTR-11, Fairfield, Conn, USA). The volume density of cells was determined as follows (Weibel, 1979):

$$V_V = y/x, \tag{2}$$

where y was the number of test points touching the cells and x was the number of test points touching the whole tissue (reference area). The numerical density of cells was calculated from the formula (Weibel, 1979):

$$N_V = N_A/(\bar{D}+t), \tag{3}$$

where N_A was the number of nuclear profiles per mm^2 of reference area; $\bar{D}$ was the mean diameter of the nucleus: this included also the shape coefficient used in this formula (Paukkonen *et al.* 1984); and t was the section thickness. For statistical tests, the Mann–Whitney U test was used. Student's t test was used, however, for testing the significance of differences in the diameters of nuclei between experimental groups. Further details of the stereological method and the validity, precision and three dimensional character of the estimators have been discussed elsewhere (Paukkonen *et al.* 1984).

RESULTS

Qualitatively, no differences were observed between physically exercised rabbits and the control group (Fig. 1). Non-strenuous exercise appeared not to cause any degenerative changes, such as fibrillation or decreased matrix stainability (Mankin, 1974) in the tibial articular cartilage of young rabbits.

Quantitative results of stereological evaluations are presented in Table 1. In the exercised group, the volume density of cells increased by 25% ($P < 0{\cdot}05$) in the middle zone (10·4% of tissue volume), by 19% ($P < 0{\cdot}05$) in the deep zone (8·0% of tissue volume) and by 22% ($P < 0{\cdot}01$) in the cartilage as a whole (9·4% of tissue volume) compared to the control group. As there was no change in numerical density of chondrocytes nor in cartilage thickness, this was due to increased cell size. After physical exercise, the mean volume of a nucleus (56×10^{-12} cm^3) was 30% ($P < 0{\cdot}05$) greater in the superficial cartilage zone of exercised rabbits than in the controls. For both exercised and control rabbits, the size frequency distribution for the diameters of nuclei showed a separate population of small nuclei in all cartilage zones (Fig. 2).

DISCUSSION

Moderate physical exercise has not been shown to result in degenerative articular changes in scanning electron microscopical (Videman *et al.* 1979; Jurvelin *et al.* 1985), biochemical (Videman *et al.* 1979; Tammi *et al.* 1983) or histological-histochemical studies (Lanier, 1946; Sääf, 1950; Kincaid & Van Sickle, 1982) on young or adult experimental animals. In histological studies of the effects of running on articular cartilage, the absence of distinct qualitative changes has necessitated the

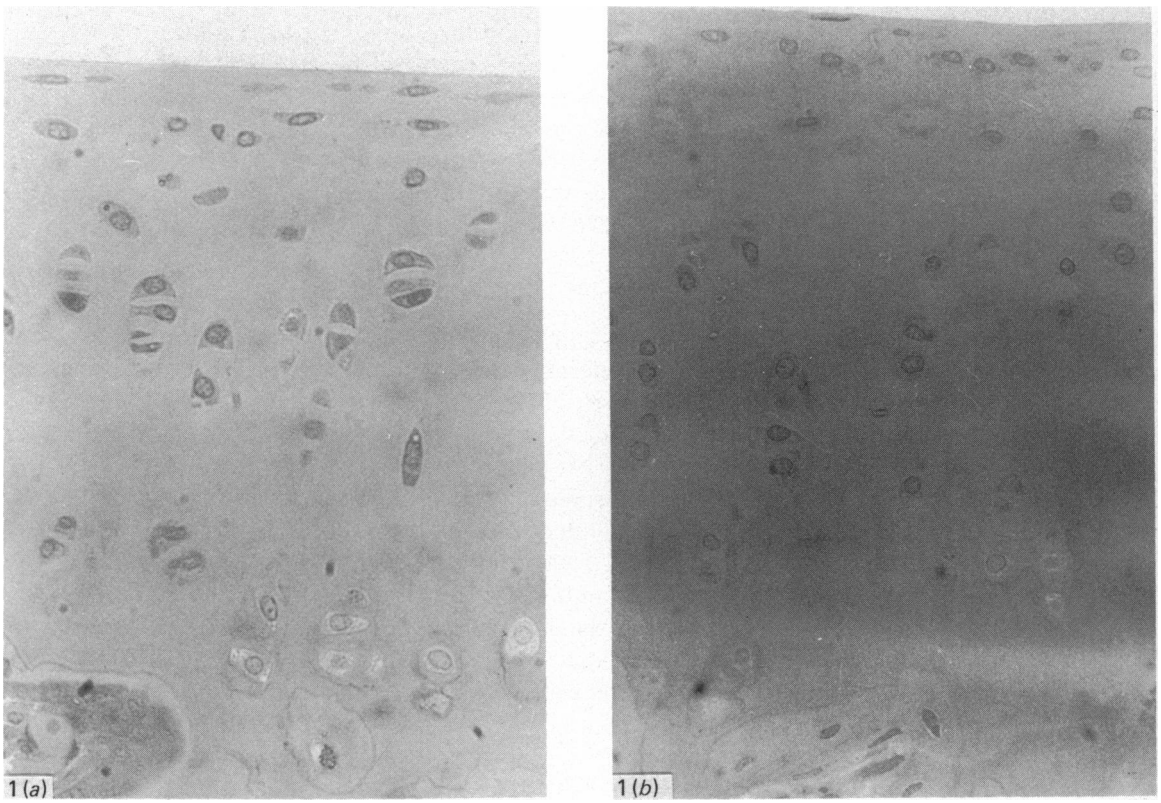

Fig. 1 (*a–b*). Tibial articular cartilage stained with iron haematoxylin and Safranin O from the knees of control (*a*) and exercised (*b*) rabbit knee. No conspicuous qualitative differences could be observed between control and exercised groups. 1 μm thick Epon sections. × 240.

use of quantitative morphological methods to discern alterations in chondrocytes and matrix. By applying quantitative methods, Sääf (1950) was able to show an increase in cell size in the articular cartilage of the humeroscapular joint of exercising adult guinea-pigs. In a previous study, Paukkonen *et al.* (1984) applied the most recent knowledge of histomorphometry (Shay, 1975; Weibel, 1979; Cruz-Orive & Weibel, 1981) to design a new stereological method that is especially appropriate for articular cartilage research. Using this method, the effects of physical exercise on the morphology of articular cartilage have now been examined in the knee joints of young rabbits.

Qualitative examination of cartilage from exercised rabbits shows that the structure and matrix stainability of the tissue are the same as in cartilage of the controls. Quantitatively, physical exercise increases the average volume of nuclei in the superficial zone and increases cell size in the middle and deep zones of articular cartilage (Table 1). Cell size is also increased in the superficial zone but, owing to the great variation in estimates, the difference is not statistically significant. No changes are observed in cartilage thickness or in the numerical density of cells. In the size frequency distribution of nuclear diameters, the separate population of small nuclei observed in both groups might represent degenerative nuclei associated with the decrease in cell density during maturition of articular cartilage (Stockwell, 1979).

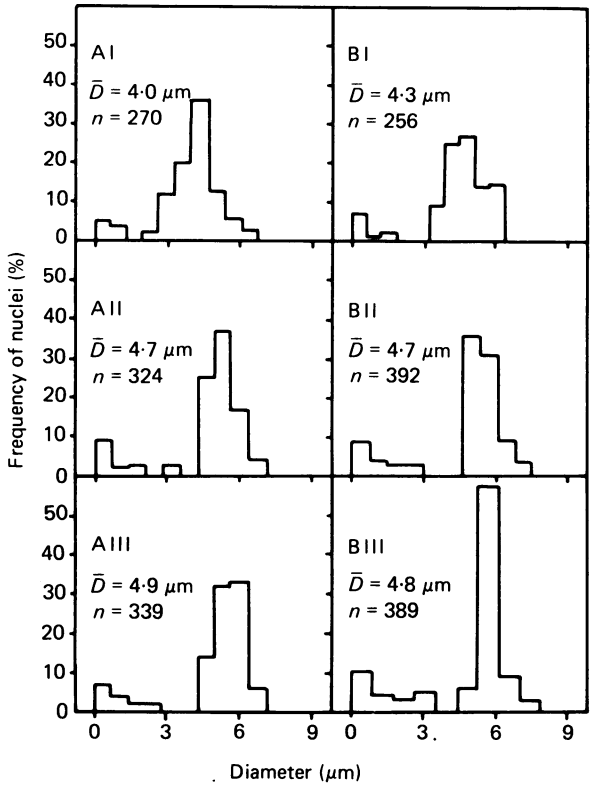

Fig. 2. Size frequency distribution of nuclear diameters in the superficial (I), middle (II) and deep (III) zones of articular cartilage in control (A) and exercised (B) groups. In the superficial zone, the average diameter of the nucleus in exercised cartilage (4·3 μm) was significantly greater ($P < 0.05$) than in the controls (4·0 μm). Note the separate populations of small nuclei in all zones of both groups. $\bar{D}$, average diameter of the nucleus; n, number of counted nuclei.

In their roentgenological study on the thickness of articular cartilage in the rabbit knee, Ingelmark & Ekholm (1948) observed a 10% increase in cartilage thickness due to swelling after the animals had been running for 10 minutes; this increase, however, disappeared within half an hour. In their light and electron microscopical study, Gritzka *et al.* (1973) found pyknosis of chondrocytes caused by continuous compression in the moving elbow joint of the rabbit. Since compression causes loss of water from cartilage, these authors suggested that the hyperosmotic matrix caused desiccation of the chondrocytes. Such loss of intracellular water would then result in pyknosis. Therefore, enlargement of chondrocytes and nuclei in exercised animals might be due either to hypertrophic activation or to osmotic swelling of the cells. Since the proteoglycan content of articular cartilage matrix tends to increase after excessive weight-bearing and physical exercise (Kostenszky & Olah, 1972; Tammi *et al.* 1983) and the reported swelling appears to be transient, it is suggested that physical exercise causes hypertrophic activation of individual cartilage cells to synthetise matrix molecules. Additional confirmation of this hypothesis has been made in preliminary studies using transmission electron microscopy of chondrocytes subjected to physical exercise (author's unpublished data). The cells contained cytoplasm rich in granular endoplasmic reticulum and other cell organelles. Since there

Table 1 *Effects of 8 weeks' physical exercise on the tibial articular cartilage of the rabbit knee joint using stereological histomorphometry*

		Control	Running
Cartilage thickness (mm)		†0·36±0·09	0·33±0·04
Volume density of cells, V_{Vc} (%) (volume of cells per tissue volume unit)	‡I	8·9±1·2	10·5±1·9
	II	8·3±1·5	10·4±1·7*
	III	6·7±1·2	8·0±0·7*
	IV	7·7±1·0	9·4±1·1**
Numerical density of cells, N_{Vc} ($\times 10^3$ per tissue mm^3)	I	282±31	280±71
	II	127±12	131±21
	III	96±13	99±15
	IV	132±10	136±13
Total volume of cells in cartilage lying beneath 1 mm^2 of articular surface, AV_{Vc} (mm^3 $\times 10^{-3}$)	I	3·5±0·5	4·2±0·8
	II	13·6±5·8	14·8±2·3
	III	10·5±3·4	11·5±1·6
	IV	27·7±7·8	30·4±3·3
Total number of cells in cartilage lying beneath 1 mm^2 of articular surface, AN_{Vc} ($\times 10^3$)	I	11·2±1·2	10·6±1·5
	II	19·4±5·6	18·6±3·3
	III	15·7±4·4	14·2±2·6
	IV	46·3±7·8	44·2±5·7
Cell size (volume) V_{Vc}/N_{Vc} (μm^3); cell diameter (μm)	I	320±50; 8·4	390±100; 9·1
	II	660±140; 10·8	800±140; 11·5*
	III	690±90; 11·1	830±150; 11·6*
	IV	580±90; 10·4	700±100; 11·0*
Volume density of nuclei, V_{Vn} (%) N_{Vc} × volume of average nucleus	I	1·2±0·13	1·6±0·39*
	II	0·9±0·08	1·0±0·15
	III	0·9±0·11	0·8±0·13
	IV	0·9±0·07	1·0±0·09
Nucleus/cell–volume ratio	I	0·14±0·02	0·15±0·04
	II	0·11±0·03	0·09±0·02
	III	0·12±0·02	0·11±0·02
	IV	0·12±0·02	0·11±0·01
Matrix volume per cell, $(1-V_{Vc})/N_{Vc}$ (μm^3)	I	3260±380	3350±680
	II	7290±690	7560±1400
	III	9820±1260	9480±1630
	IV	7030±540	6730±680
Volume of average nucleus (μm^3); §diameter of average nucleus (μm)	I	43; 4·0±1·4	56; 4·3±1·5*
	II	71; 4·7±1·8	74; 4·7±1·8
	III	82; 4·9±1·8	82; 4·8±2·0
	IV	68; 4·6±1·7	73; 4·6±1·8

† Values of estimates are presented as means±S.D.
‡ I, Superficial zone; II, middle zone; III, deep zone; IV, whole cartilage.
§ Student's *t* test was used only for this parameter.
* $P < 0·05$, ** $P < 0·01$ as compared to control (Mann–Whitney U test).

was no change in cartilage thickness, these results imply that either the cartilage matrix grows denser or the matrix turnover is accelerated.

In conclusion, non-strenuous physical exercise causes no qualitative changes in articular cartilage. The most significant quantitative changes in articular cartilage structure are the increased size of the chondrocytes in the middle and deep zones as well as an increased size of nuclei in the superficial zone, indicating (probable) hypertrophic activation of the chondrocytes.

SUMMARY

Effects of non-strenuous physical exercise on the articular cartilage in the knee joints of young rabbits (4·5 to 5 months old) were investigated by quantitative histology, after an 8 weeks' period during which the rabbits ran daily. Using stereological methods, three dimensional parameters were derived for defining the tissue structure. Physical exercise increased the size of chondrocyte nuclei and cells; cell size increased especially in the middle and deep zones of articular cartilage. Neither cartilage thickness nor cell number increased in the experimental animals as compared to the controls. No signs of degeneration were observed in the exercised cartilage. The increase in cell size in exercised articular cartilage indicates hypertrophic activation of the chondrocytes.

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