Upsala J Med Sci 90: 243–258, 1985

Studies of the Lumbar Vertebral End-plate Region in the Pig

Marianne Törner and Sten Holm

Department of Orthopaedic Surgery I, University of Göteborg, Sahlgren Hospital, Göteborg, Sweden

ABSTRACT

The vertebral end-plates and the epiphyses from the lumbar region of the spine in juvenile pigs were studied. Cell density of the hyaline cartilage of the vertebral bodies was measured as well as cartilage thickness.

The relative bone content in the region of the nucleus pulposus was calculated.

A certain negative interrelationship between cartilage thickness and cell density of the hyaline cartilage of the vertebral bodies was found.

The relative bone content of the cranial epiphysis was higher than that of the caudal epiphysis in the same motion segment at all levels of the lumbar spine. The difference was pronounced down to 2 mm from the cartilage/bone interface.

INTRODUCTION

The vertebral body consists of a core of cancellous bone surrounded by a thin layer of cortical bone (3). In each vertebral body there are also the two epiphyseal growth plates and the epiphyses. The epiphyses consist of cancellous bone distally covered by hyaline articular cartilage; the end-plates, forming an interface between the bone and the intervertebral discs.

The cancellous bone of the epiphysis is vascularized by capillaries and the blood reaches the bone/cartilage interface through a rich sinusoidal network (3.4). It has previously been demonstrated that the central area of the distal part of the epiphysis (adjacent to the nucleus pulposus and the inner annulus fibrosus of the disc) of the human adult lumbar vertebrae is supplied with nutrients via the nutrient and metaphyseal arteries, whereas the peripheral area of the distal part of the epiphysis (adjacent to the outer ring of the annulus fibrosus) is supplied via the peripheral arteries (18). It has also been

suggested that the peripheral arteries are less subject to arterial degeneration than the nutrient and metaphyseal arteries (18).

Brookes (1977) stated that the arterial blood flow to the vertebrae and the vascularisation of the entire vertebral body is rich, which suggests that the oxygen tension of the blood here is relatively high. Brookes concluded, that this high oxygen tension was probably linked to the haematopoietic function of the bone-marrow of the vertebrae and the dominating trabecular architecture of the bone within the vertebral bodies (3). The vascularisation of the vertebral bodies has also been qualitatively described by others (4,5,8,18,20,21).

The intervertebral discs are situated between the vertebral bodies. The disc is the largest avascular tissue of the body and is for its nutrition completely dependent upon nutrients entering from surrounding blood pools.

It has previously been demonstrated that there are two main nutritional routes into the intervertebral disc, i.e. transport

a) from the blood vessels surrounding the periphery of the annulus fibrosus, and
b) through the end-plates (predominantly in the region of the nucleus pulposus)(2,6,11,16,18).

The nutritional status of the cells of the central nucleus pulposus is precarious; the oxygen tension field as well as the concentration gradient of glucose is very steep and the cells in the central region of the tissue may not be able to satisfy their requirements (10). Cell necrosis in the disc due to lack of substrates such as oxygen and glucose may lead to severe disc degeneration. It has been suggested that one reason for changes in the discal nourishment may be changes in the vertebral body blood supply (18).

Certain features of the epiphysis govern factors that are of special importance for the nutritional status of the central part of the disc:

1) The arterial blood that reaches the vertebrae enters the blood pools from the sinusoidal network of the epiphysis. Nutrients diffuse into the marrow-spaces and extra cellular liquid in the cancellous bone of the epiphysis and further from these spaces into the disc. The amount of blood pools and marrow-spaces in the immediate vicinity of the bone/cartilage interface is therefore of importance to the transport of solutes to the cells of the disc. The route of diffusion via the peripheral part of the annulus may for certain solutes be too long to contribute to any considerable extent.

2) Nutrients must, in order to reach the disc, diffuse through the hyaline cartilage of the end-plate. The cell density of this cartilage is much higher than that of the central part of the disc. This relation has been established to be approximately 2.8:1 (10). A certain amount of nutrients leaving the bone will therefore be consumed by the chondrocytes of the hyaline cartilage.

Hence, the thickness and cell density of this cartilage is of importance to the nutritional status of the cells of the disc.

Ingelmark and Ekholm (1948) reported that the thickness of the hyaline cartilage of the knee joint in the rabbit varies with physical exercise. They observed a 10-12% increase in cartilage thickness after 10 minutes of dynamic exercise (running), compared to the cartilage thickness after 60 minutes of rest in a position where the knee joint was not stressed. It was stated that this rapid increase in cartilage thickness was due to an increase in fluid content of the cartilage (12), and that flow of fluid from the marrow cavities gave higher controbution than from the articular space (12). No such increase in cartilage thickness with physical exercise has been reported concerning the hyaline cartilage of the vertebral bodies.

Bernick and Caillet (1982) observed age changes in the arterioles, capillaries and venules in the nutrient canals or spaces of the bone adjacent to the cartilage or disc in human lumbar vertebrae. They also observed that the articular cartilage of the end-plate undergoes calcification followed by resorption and replacement by bone with increasing age. They stated that this calcification of the articular cartilage and the vascular changes seen in the older vertebrae would inpede the passage of nutrients from the blood to the disc (1).

3) Since one of the main transport mechanisms of solutes to the cells of the disc is diffusion through the end-plates, the distance between the vertebral body/disc interface and the central disc is of importance.

The aim of this investigation was to study the above mentioned features in the end-plate region.

MATERIALS AND METHODS

Seven juvenile pigs of both sexes (9-10 months old, average weight 63 kg) were included in the investigation. Initially, an intramuscular injection of Ketalar (Parke-Davis, Detroit, Mich., USA) was administered. After that a catheter was inserted into a vein of the ear and an overdose of Pentothal sodium (Abbot Laboratories, Chicago, Ill., USA) with KCl was administered to kill the animal.

The entire lumbar spine together with the major part of the thoracic spine was excised, quickly freed of soft tissue, frozen - and subsequently stored - at $-20^{\circ}C$.

The spine was later, while being kept frozen, sectioned by an electrically powered bandsaw into vertebral motion segments, each motion segment consisting of one intervertebral disc and half the vertebral bodies immediately cranially

and caudally to it, (Figure 1). The motion segments were then cut in half by a medial sagittal cut.

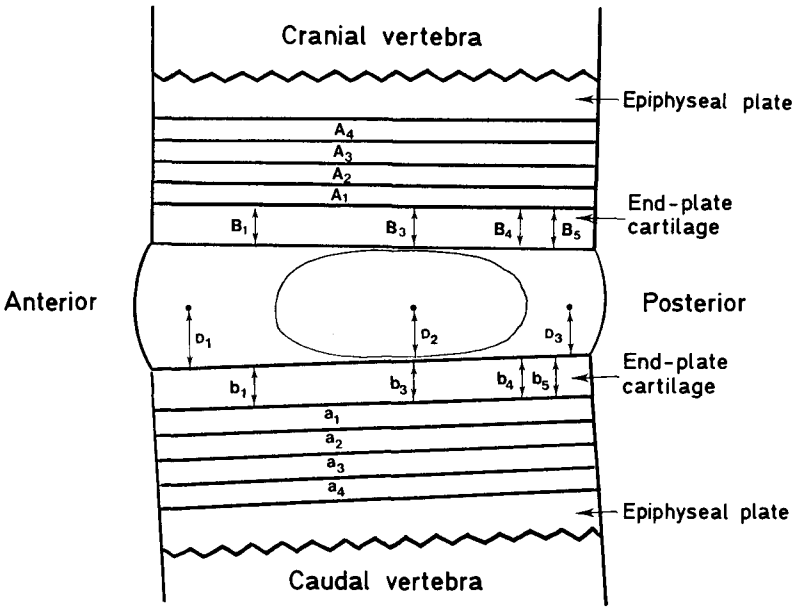

Fig. 1. Schematic drawing of a motion segment consisting of one intervertebral disc and half the vertebral bodies immediately cranially and caudally to it. The drawing shows sections indicated as A_1-A_4 and a_1-a_4: strips of the epiphysis (0.5 mm wide) where relative bone content was measured.

B_1-B_5 and b_1-b_5: sites in the hyaline end-plate cartilage where cell density and cartilage thickness were measured. The symbols indicate sites adjacent to the following regions of the intervertebral disc:

B_1 and b_1: Anterior inner annulus fibrosus.
B_3 and b_3: Central nucleus pulposus.
B_4 and b_4: Posterior peripheral part of the nucleus pulposus.
B_5 and b_5: Posterior inner annulus fibrosus.

D_1 - D_3: Sites where disc thickness was measured.

The dimensions of the discs were measured macroscopically (Fig: 1: D_1, D_2, D_3, and Fig. 2: E, F, G) and each half motion segment was thereafter cut sagittally into 2-3 mm thick slices. During this entire procedure the specimens were kept frozen by dry ice. Some of the slices were fixed in 10% buffered formalin (15,17) for 5-7 days after which they were left for 3-5 days in formic acid for decalcification (17). A rectangular piece with the approximate dimension of 10 x 15 mm was cut out around the nucleus pulposus of the disc in

each decalcified slice (Fig.2). This piece was embedded in paraffin wax and sectioned further into 5 μm thin slices on a sliding microtome. The slices were then strained in haematoxylin/eosin (9,14).

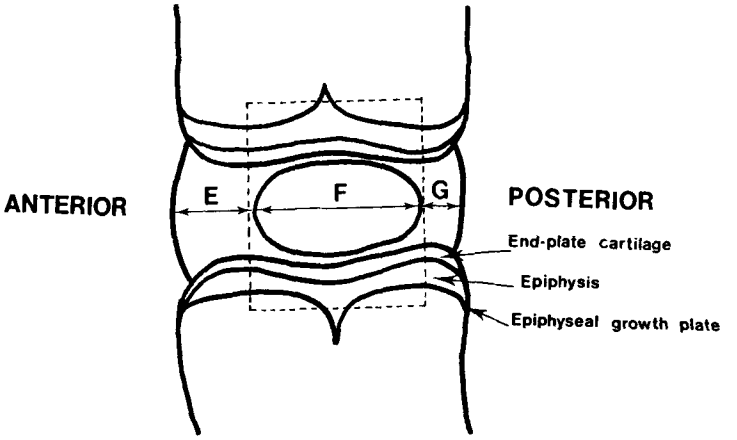

Fig. 2. Vertebral motion segment. E, F, and G show measured anterior-posterior widths of the intervertebral disc. The dashed line indicates the area around the nucleus pulposus cut out of the decalcified slices for further histological studies.

The thickness of the hyaline cartilage of the end-plate was measured and the amount of cells per area in cartilage was counted by using a squared graticule. This procedure was performed under a photomicroscope (Zeiss photomicroscope II) at x320 magnification . The measurements were performed at different spinal levels. The measuring sites are indicated in Fig. 1 (B_1 - B_5 and b_1 - b_5).

Central sections of two motion segments (L3/L4) from different pigs were photographed through the photomicroscope in an overlapping manner at x50 magnification. The pictures were then mounted together to obtain a complete view of the magnified motion segment. A plastic sheet with the known average weight of 0.01328 g/cm^2 was fixed over the picture of each epiphysis of the motion segment.

All marrow-spaces were drawn on the plastic sheet, which was divided into parallell strips corresponding to 0.5 mm of the vertebral tissue (a_1 - a_4 in Fig 1). Areas in each strip, corresponding to marrow-spaces and bone trabeculae, were separated and weighed. Their respective areas were calculated.

The results of the above described procedure are presented as mean values where standard deviations and number of included measurements are given.

Methodological problems and errors

1. Measurements of cartilage thickness.

The interface between bone and hyaline cartilage is not straight and sharply defined due to the rich vascular sinusoidal network of this area (Fig. 3). Here an arbitary line had to be drawn. However, the measurements were at a fairly great magnification (x320) and therefore the resolution was good, which simplified the determination of the position of the interface. The error was approximately ±10%.

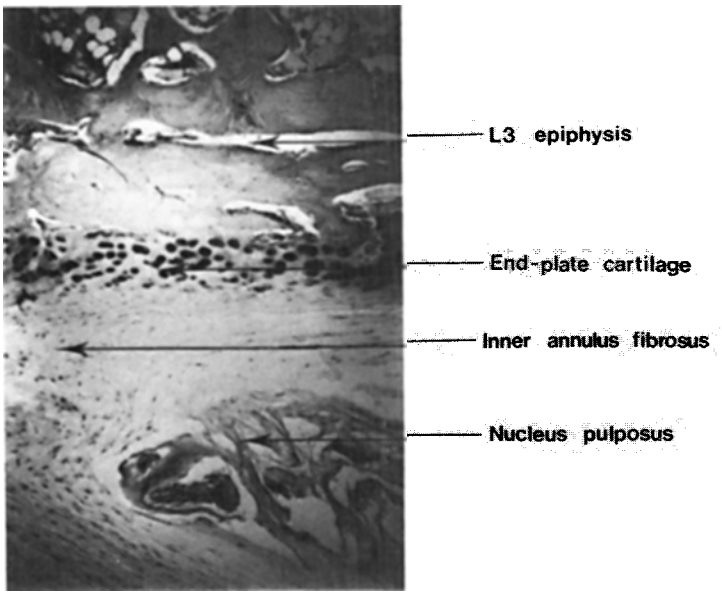

Fig. 3. Part of vertebral motion segment showing posterior peripheral nucleus pulposus and inner annulus fibrosus, hyaline end-plate cartilage and the cancellous bone of the L3 epiphysis with the uneven bone-cartilage interface due to the sinusoidal vascular network of the vertebra. (Photo-microscope x50).

2. Measurements of cell density.

Even at a considerable magnification it is sometimes difficult to decide whether what is seen under the microscope is actually a chondrocyte, just an empty lacunae or possibly two cells in the same lacunae. However, as a large number of measurements were made, the magnitude of this kind of error was minimized to approximately ±15%.

3. Measurements of bone content.

The estimation of the area and the subsequent weighing procedures were done manually. In this method there are several sources of possible errors. The resolution at a magnification of x50 is good, but not absolute. The ink of the pen with which the marrow-space were drawn on the plastic sheet has a certain weight which has been neglected. However, the method is simple, but nontheless quite accurate. The entire procedure of determining bone content was repeatedly performed and the reproducibility was found to be acceptable. The error averaged ±2%.

RESULTS

1. Macroscopic dimensions of the intervertebral discs of the pig

The anterior-posterior width of the disc varies between different levels of the lumbar spine. In this study the mean disc width at the Th15/L1 level was 18.56 mm. The width then gradually increased at the L1/L2 and L2/L3 levels. The highest mean value, 21.05 mm, was obtained at the L3/L4 level. A further descent of the lumbar spine showed a decreasing disc width and the lowest mean value of the entire lumbar spine, 17.78 mm, was found at the L5/L6 level (Table 2).

The caudal-cranial distance from the cartilage/disc interface to the central disc did not vary significantly between different levels of the lumbar spine. However, our results indicated that the central part of the disc was somewhat thicker at the Th15/L1 and L5/L6 levels, 1.92 mm and 2.19 respectively (Table 1).

Table 1. Average distance (mm) from the surface of the end-plate cartilage to the center of the disc at different levels of the lumbar spine. The sites of measurement indicated as D_1, D_2 and D_3 are shown in Figure 1.

		MOTION SEGMENT					
Site of measurement		Th15/L1	L1/L2	L2/L3	L3/L4	L4/L5	L5/L6
D_1	Mean	1.58	1.43	1.39	1.44	2.13	1.31
	S.D.	0.58	0.45	0.28	0.33	1.45	0.25
	N	6	4	5	3	4	4
D_2	Mean	1.90	2.14	1.95	2.20	2.15	2.10
	S.D.	0.12	0.53	0.20	0.26	0.32	0.16
	N	6	3	5	3	4	4
D_3	Mean	1.00	0.98	0.94	1.05	1.01	1.00
	S.D.	0.45	0.10	0.20	0.17	0.19	0.18
	N	6	4	5	3	4	4

Table 2. The anterior-posterior width (mm) of the intervertebral discs at different levels of the lumbar spine. The sites of measurement indicated as E, F and G are shown in Figure 2.

	SAGITTAL WIDTH OF DISC			
	Annulus Anterior	Nucleus	Annulus Posterior	Total Width
Motion segment	E	F	G	E+F+G
Th15/L1	5.35	11.65	2.10	19.10
	2.35	13.05	1.95	17.35
	3.00	12.95	2.35	18.30
	3.35	11.05	2.05	16.45
	3.80	11.25	3.10	18.15
	6.10	12.80	3.10	22.00
Mean	3.99	12.12	2.44	18.56
S.D.	1.44	0.91	0.53	1.91
L1/L2	6.10	13.75	2.20	22.05
	2.60	14.00	2.40	19.00
	3.70	13.40	2.60	19.70
	2.90	11.10	2.75	16.75
Mean	3.82	13.06	2.49	19.38
	1.59	1.33	0.24	2.18
L2/L3	6.95	11.10	3.50	21.55
	3.65	14.25	2.70	20.60
	4.00	13.20	2.85	20.05
	3.90	11.20	2.85	17.95
	3.80	11.95	3.40	19.15
Mean	4.46	12.34	3.06	19.86
S.D.	1.40	1.36	0.36	1.38
L3/L4	7.05	12.00	2.80	21.85
	3.80	11.25	1.85	16.90
	9.20	12.10	3.10	24.40
Mean	6.68	11.78	2.58	21.05
S.D.	2.72	0.46	0.65	3.81
L4/L5	3.25	13.65	2.60	19.50
	4.15	10.80	2.20	17.15
	4.50	11.70	2.70	18.90
	3.90	11.70	2.00	17.60
Mean	3.95	11.96	2.38	18.29
S.D.	0.53	1.20	0.33	1.10
L5/L6	3.50	13.35	2.10	18.95
	4.85	8.90	2.45	16.20
	4.70	11.10	3.20	19.00
	4.50	10.65	1.80	16.95
Mean	4.39	11.00	2.39	17.78
S.D.	0.61	1.83	0.60	1.42

2. Microscopic dimensions of the end-plate

a) Cartilage thickness and cell density of the end-plate

The thickness of the end-plate cartilage was lower at the Th15/L1 level than at the L3/L4 level (Table 2). At the Th15/L1 level the central part of the end-plate cartilage, adjacent to nucleus pulposus, was thinner than the peripheral part adjacent to the annulus fibrosus (Table 3). At the same spinal level the cell density was higher centrally than peripherally.

At the Th15/L1 level the cartilage was peripherally somewhat thicker in the caudal end-plate than in the cranial end-plate of the same motion segment, whereas the cell density was higher in the cranial than in the caudal end-plate (Table 3). At the L3/L4 level the opposite relation was seen; the cartilage was thicker in the cranial end-plate and the cell density was lower (at least centrally and posteriorly) than in the caudal end-plate of the same motion segment (Table 3).

Cell density of the hyaline cartilage was plotted versus cartilage thickness (Fig. 5a and 5b), but the correlation coefficient was low ($r^2<0.56$), when fitting the values to a curve, linear or non-linear.

b) Bone content of the epiphyses

The thickness of the different epiphyses varied between 1.5-2.2 mm. The relative bone content of the cranial epiphyses in the region of the nucleus pulposus was higher than that of the caudal epiphyses in the same motion segment at all levels of the lumbar spine (Fig. 4a and 4b). The difference was conciderable in the sagittal area 0-1.5 mm from tha cartilage/bone interface. At the area 0-0.5 mm from this interface the difference in bone content of the two epiphyses was approximately 20%, at the area 0.5-1.0 mm the difference in one case was 11% and in the other 32% and at the area 1.0-1.5 mm from the interface the difference was 7-8%. No difference in bone content of the epiphyses of one motion segment was registered at the area 1.5-2.0 mm from the cartilage/bone interface (Fig 6).

Table 3. Average cartilage thickness (mm) and cell density (cells/mm^2) of the hyaline end-plate cartilage at three different spinal levels.

	ANNULUS (anteriorly) END-PLATE		NUCLEUS END-PLATE		ANNULUS (posteriorly) END-PLATE		
	Cranial	Caudal	Cranial	Caudal	Cranial	Caudal	Motion segment
Cartilage thickness ($x10^{-1}$mm)	1.88	1.95	1.84	1.82	1.93	2.81	
N	3	2	10	6	3	1	
S.D.	0.54		0.43	0.21	0.39		Th15/L1
Cell density ($x10^2$ cells/mm)	6.930	4.506	9.408	8.667	6.562	5.900	
N	3	2	10	6	4	2	
S.D.	2.30		2.48	1.71	1.56		
Cartilage thickness	1.56	1.72	1.46	1.51	1.80	1.72	
N	1	1	3	3	2	2	
S.D.			0.36	0.09			L2/L3
Cell density	8.602	8.006	11.563	12.720	11.776	8.472	
N	1	1	4	3	1	2	
S.D.			6.16	1.72			
Cartilage thickness	2.28	2.08	2.50	2.22	2.41	2.07	
N	3	3	5	8	3	3	
S.D.	0.75	0.48	0.31	0.31	0.66	0.37	L3/L4
Cell density	9.365	7.212	9.404	11.427	8.350	11.100	
N	2	7	2	6	2	2	
S.D.		4.36		2.43			

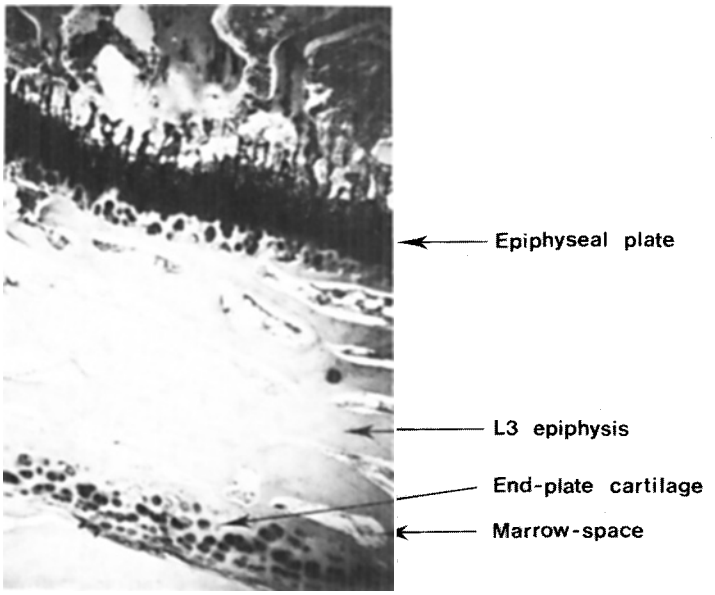

Fig. 4a. Section of the L3 vertebral body from the area adjacent to the central nucleus pulposus. Notice the dence cancellous bone, with small marrow-spaces, of the epiphysis. (Photo-microscope x50).

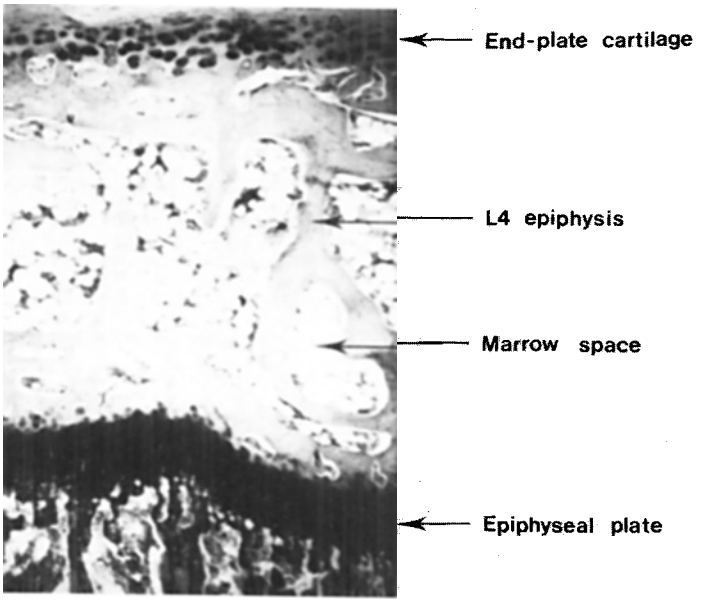

Fig. 4b. Section of the L4 vertebral body from the region adjacent to the central nucleus pulposus, showing big marrow-spaces and low bone content of the epiphysis.

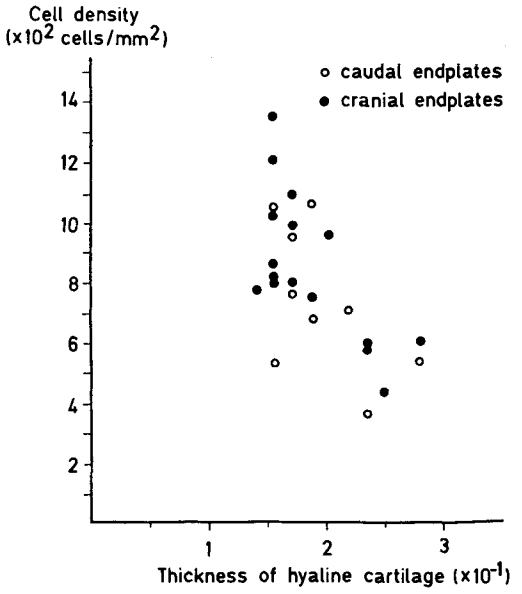

Fig. 5a. Cell density (cells/mm^2) of the Th15/L1 end-plate cartilage versus cartilage thickness (mm).

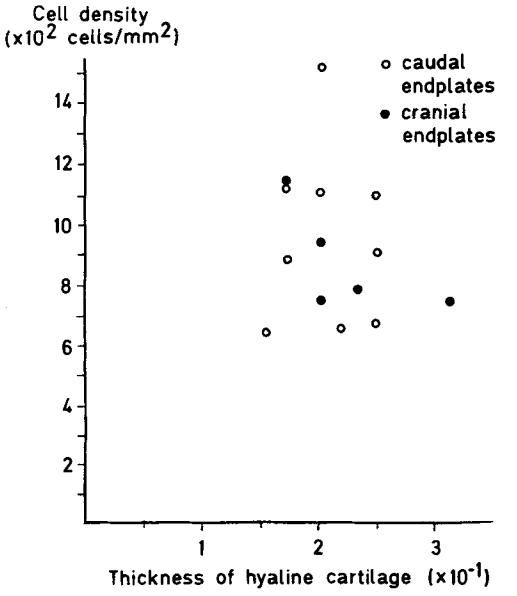

Fig. 5b. Cell density (cells/mm^2) of the L3/L4 end-plate cartilage versus cartilage thickness (mm).

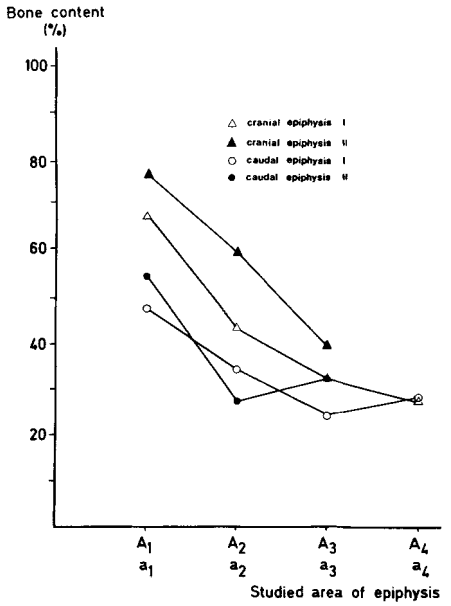

Fig. 6. Relative bone content of the epiphysis in the region adjacent to the nucleus pulposus at the L3/L4 level, expressed as a percentage of the total sagittal area.

DISCUSSION

The hyaline cartilage of the end-plates in normal human adult subjects is avascular. The cells of hyaline cartilage are chondrocytes and each group of cells is surrounded by a capsule rich in glycosaminoglycans (13). In the region of the nucleus pulposus the nuclei of the chondrocytes located in the basal two thirds of the end-plate cartilage, adjacent to the bone, are rounded and the chondrocyte capsules stain darkly in haematoxylin/eosin. The nuclei of the chondrocytes located in the superficial third of the cartilage in this region, adjacent to the nucleus pulposus, as well as the ones located in the peripheral part of the end-plate in the region of the annulus fibrosus, are cigar shaped. The equally cigar shaped chondrocyte capsules in these regions are smaller than the ones described earlier and stain lightly in haematoxylin/eosin.

The findings of a negative interrelationship between cartilage thickness and cell density of the articular cartilage of the end-plate are in agreement with the results of Stockwell in his study of articular cartilage (19), although the correlation coefficient of this interrelationship in the present study was low.

The difference in bone content between the two epiphyses of one motion segment was measured in two different samples, both at the L3/L4 level.

This difference was observed also in all other sections, from different levels of the lumbar spine. When studying a motion segment under the microscope it could easily be determined (from the sole criterion of bone content), which epiphyses was the caudal one and which was the cranial one. The same observations were made when studying sections of motion segments from dog (unpublished results).

It has previously been suggested that bone mineral content determined by dual photon absorptiometry is higher in the cranial than in the caudal part of one motion segment in man (7). It is also an impression that healing microfractues in human vertebrae predominantly have been found in the caudal epiphysis of a motion segment (7). These circumstances together with our findings of a lower relative bone content in the caudal than in the cranial epiphysis of one motion segment indicate that the caudal epiphysis is more sensitive to the effects of demineralization than the cranial epiphysis. This would be the case already in a juvenile and healthy individual.

The difference in relative bone content was observed to a depth of 1.5 mm measured from the articular cartilage. At greater depths there was no noticable difference. However, blood vessels and blood pools at greater depths than approximately 1.5 mm are probably of little importance as far as diffusion of nutrients to the disc is concerned.

A lower relative bone content in the caudal epiphysis of a motion segment obviously means a higher relative content of marrow spaces. These spaces contain the blood vessels and blood pools on which the disc is highly dependent for its nourishment. A microfracture in this epiphysis might therefore have consequences not only for the vertebra itself, but also impair the transport of nutrients to the intervertebral disc, something which might be deleterious to the cells of the nucleus pulposus.

ACKNOWLEDGEMENTS

These studies were supported by grants from the Göteborg's Läkaresällskap, Riksförbundet mot Rheumatism and the Swedish Medical Research Council (5979).

REFERENCES

1. Bernick, S. & Caillet, R: Vertebral end-plate changes with aging of human vertebrae. Spine 1982;7:2:97-102.

2. Brodin, H: Paths of nutrition in articular cartilage and intervertebral discs. Acta Orthop Scand 1955;24:177-80.

3. Brookes, M: The blood supply of bones. Butterworths, London 1971:47.

4. Farfan, H: Mechanical disorders of the low back. Lea and Febiger, Philadelphia 1973.

5. Guida, G. Cigala, F. & Riccio, V: The vascularisation of the vertebral body in the human foetus at term. Clin Orthop and Rel Res 1969;65:229-234.

6. Hansen, HJ. & Ullberg, S: Uptake of ^{35}S in the intervertebral disc after injection of ^{35}S-sulphate. An autoradiographic study. Acta Orthop Scand 1961; 30:84-90.

7. Hansson, T: Dept of Orthop Surg I, Sahlgren Hospital, University of Göteborg, Sweden. Personal communication.

8. Harris, RS. & Jones, DM: The arterial supply to the adult cervical vertebral bodies. J of Bone and Joint Surg 1956;38-B:922-7.

9. Harrison, RC. & Gamez, FN: Hormonal effects on the vascularisation of bone. Symp of the Zool Soc, London 1964;II:I.

10. Holm, S: Nutrition of the intervertebral disc. Transport and metabolism. Thesis, Dept of Orthop Surg I, Sahlgren Hosp, University of Göteborg, Göteborg, Sweden 1980.

11. Holm, S., Maroudas, A., Urban, JPG., Selstam, G. & Nachemson, A: Nutrition of the intervertebral disc: Solute transport and metabolism. Connective Tissue Research 1981;8:101-19.

12. Ingelmark, BE. & Ekholm, R: A study on variations in the thickness of articular cartilage in association with rest and periodical load. Uppsala Läkareförenings Förhandlingar 1948;53:61-74.

13. Junqueira, LC. & Carneiro, J: Basic histology. Lange Medical Publications 1971:122-7.

14. Kelly, PJ., Peterson, LFA. & Janes, JM: A method of using sections of bone prepared for microangiography for subsequent histologic study. Proceedings of the staff meetings, Mayo Clinic 1959b;34:274-83.

15. Kelly, PJ. & Janes, JM: Microangiographic and histologic studies of vascular anatomy of the femour and tibia distal to femor and iliac arteriovenous fistulas in dogs. Anatomical Record 1968;162:255-67.

16. Nachemson, A., Lewin, T., Maroudas, A. & Freeman, MAR: In vitro diffusion of dye through the end-plates and the annulus fibrosus of human lumbar intervertebral discs. Acta Orthop Scand 1970;41:589-607.

17. Peterson, LFA, Kelly, PJ. & Janes, JM: Ultrastructure of bone: Technique of microangiography as applied to the study of bone: Preliminary report. Proceedings of the staff meetings, Mayo Clinic 1957;32:681-6.

18. Ratcliffe, JF: The arterial anatomy of the adult human lumbar vertebral body: A microarteriographic study. J of Anatomy 1980;131(1):57-79.

19. Stockwell, RA: The interrelationship of cell density and cartilage thickness in mammalian articular cartilage. J of Anatomy 1971;109(3):411-21.

20. Wagoner, G. & Pendergrass, EP: Intrinsic circulation of the vertebral body with roentgenologic considerations. Am J of Roentgenology 1932;27:818-26.

21. Willis, TA: Nutrient arteries of the vertebral bodies. J of Bone and Joint Surg 1949,31-A:538-40.

Address for reprints:

Marianne Törner
Department of Occupational Orthopaedics
Sahlgren Hospital
Guldhedsgatan 19
S-413 45 GÖTEBORG, SWEDEN