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Nutrition of the Intervertebral Disc

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Study Design. A review of the literature on disc nutrition.

Objectives. To summarize the information on disc nutrition in relation to disc degeneration.

Summary of the Background Data. The disc is avascular, and the disc cells depend on diffusion from blood vessels at the disc's margins to supply the nutrients essential for cellular activity and viability and to remove metabolic wastes such as lactic acid. The nutrient supply can fail due to changes in blood supply, sclerosis of the subchondral bone or endplate calcification, all of which can block transport from blood supply to the disc or due to changes in cellular demand.

Methods. A review of the studies on disc blood supply, solute transport, studies of solute transport in animal and human disc *in vitro*, and of theoretical modeling studies that have examined factors affecting disc nutrition.

Results. Small nutrients such as oxygen and glucose are supplied to the disc's cells virtually entirely by diffusion; convective transport, arising from load-induced fluid movement in and out of the disc, has virtually no direct influence on transport of these nutrients. Consequently, there are steep concentration gradients of oxygen, glucose, and lactic acid across the disc; oxygen and glucose concentrations are lowest in the center of the nucleus where lactic acid concentrations are greatest. The actual levels of concentration depend on the balance between diffusive transport and cellular demand and can fall to critical levels if the endplate calcifies or nutritional demand increases.

Conclusions. Loss of nutrient supply can lead to cell death, loss of matrix production, and increase in matrix degradation and hence to disc degeneration.

Key words: oxygen concentration, glucose concentration, lactic acid concentration, pH, diffusion, cell density.
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The intervertebral disc is the largest avascular tissue in the body, and maintenance of an adequate nutrient supply has long been regarded as essential for preventing disc degeneration.[1,2] Essential nutrients such as oxygen and glucose and substrates for matrix production such as amino acids and sulfate are supplied to the disc by the blood supply at the disc's margins[3] (Figure 1). These nutrients then move from the surrounding capillaries through the dense extracellular matrix of the disc to the cells, which, in the center of the nucleus in an adult human lumbar disc, may be 7 to 8 mm from the nearest blood supply. Metabolic waste products are removed from the tissue by the reverse route.[4] The nutritional environment of the cells thus varies throughout the disc, with the oxygen concentration and pH around cells in the disc center being different from that around cells at the disc periphery.[4] Here we will briefly review the evidence relating loss of nutrient supply to the development of disc degeneration, discuss *in vivo* and *in vitro* studies on nutrient transport, outline the factors that regulate the transport of nutrients and other molecules to and from the cells of the disc, and show how use of mathematical models can give insight into factors regulating disc nutrition.

Epidemiological Evidence

There is strong evidence that a fall in nutrient supply is associated with disc degeneration. The nutrient supply to the nucleus cells can be disturbed at several points. Disorders that affect the blood supply to the vertebral body such as atherosclerosis of the abdominal aorta are associated with disc degeneration and back pain.[5,6] There is some evidence that the capillaries of the endplate may be blocked as the result of thrombophilic and hypofibrinolytic disorders such as sickle cell anemia,[7] Caisson disease, and Gaucher disease[8]; these all appear to lead to a significant increase in adverse changes in the vertebral body and disc degeneration. Short-term exposure to vibration[9] or smoking[10] appears to inhibit nutrient transport possibly through regulation of capillary flow by muscuranic receptors.[11] Although the chronic effects of exposure to these stimuli on disc nutrition was not measured, both are associated with disc degeneration.[12,13] Apart from disturbances of the blood supply, nutrients may not reach the disc cells if there is sclerosis of the subchondral bone or if the cartilaginous endplate calcifies[14]; intense calcification of the endplate is seen in scoliotic discs, for instance. Furthermore, the permeability of the subchondral bone-cartilaginous endplate was significantly lower in degenerate discs.[2] Further evidence for disturbances of transport in degenerate discs are the high levels of lactic acid and acid pH values measured in degenerate discs.[15,16] Direct measurements have shown that transport of magnetic resonance imaging (MRI) contrast media into the disc was inhibited in early degeneration,[17] whereas in more degenerate discs, there were noticeable disturbances of the endplate region. Loss of transport of a gaseous tracer into scoliotic discs correlated with loss of cell viability.[18,19] Thus, the association between disc degeneration and disturbances to nutrient supply are strong. Whether loss of supply actually causes disc degeneration, however, is not proven. In relation to

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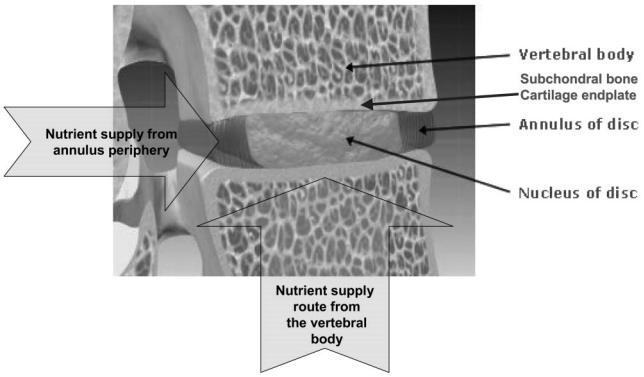

Figure 1. Schematic view of the routes supplying nutrients to the avascular intervertebral disc. Adapted from: http://www.nucleoplasty.com/dpat/dpat.aspx?s = 0201.

endplate calcification, it is not clear whether calcification preceded or followed degeneration. It seems apparent, however, that some cases of disc degeneration arise directly because a fall in blood supply to the disc limits nutrient supply.

■ *In Vivo* Observations of Nutrient Transport Into the Disc

Animal Studies

The earliest studies of transport into the disc were carried out *in vivo* with fluorescent or radioactive tracers where the tracer was injected into the animal, the animal was killed, and the disc examined. Such studies showed clearly that the routes of tracer penetration into the disc were from the vertebral body and from the tissue surrounding the anulus periphery.[20] Ageing was also found to affect transport into the disc with transport into rabbit discs falling as the animals matured and aged.[21] *In vitro* work[22] confirmed that transport was slow and also showed that disc shape and loading influenced the rate of transport.[23–25]

Tracer studies have also shown that the changes to the blood supply have a strong influence on transport of small solutes in the disc. In a series of studies, Holm and Nachemson investigated effects of both acute and chronic changes on disc nutrition. They demonstrated that exposure to cigarette smoke rapidly inhibited both transport of oxygen into the disc and escape of lactic acid from it because smoke constricted the microcirculation that supplied nutrients to the disc.[10] They showed that although short-term exercise (6 hours) had no effect on transport into the disc,[26] long-term exercise (3 months) significantly increased rates of sulfate and oxygen transport into the disc, possibly because of remodeling of the microcirculation at the vertebral body–disc interface.[27] Spinal fusion, on the other hand, had little effect on transport, although it decreased cellular activity significantly.[28]

Animal studies also showed that the degree of solute penetration depended on the characteristics of the solute, with both solute size and charge affecting transport. Cations were shown to penetrate to a greater extent than anions, as expected from the polyanionic nature of the disc matrix,[29] whereas, because of the low porosity of normal disc, large solutes such as albumin and lysozyme were virtually excluded from the disc.[30] The relative exclusion of anionic solutes from the disc has important implications because negatively charged antibiotics such as penicillin and cefuroxime have been found to penetrate far less effectively into the disc than positively charged antibiotics such as gentamycin and aminoglycosides.[31–33]

Magnetic Resonance Imaging Studies

In recent years, development in MRI has allowed almost noninvasive investigations of transport into the disc in animals and humans *in vivo*. Transport is determined from the increase in signal intensity in the disc following intravenous injection of paramagnetic medium; movement of contrast medium into the disc gives an indication of transport pathways. Charge of the contrast medium, age of the animal, and time after injection all affected penetration,[34,35] in line with results from radioactive or fluorescent tracers. For example, uncharged gadoteridol moved into the disc more readily than negatively charged contrast agents such gadopentetate because negatively charges solutes are excluded by matrix charge.[36] Magnetic resonance imaging studies have also been used to show that degree of penetration depends on solute molecular weight; coupling contrast media to a high molecular weight solute inhibited penetration.[37] Because of the noninvasive nature of MRI, it has been possible to undertake longitudinal studies on transport into dog discs after experimental maneuvers such as discectomy[38]; in this case, a change in signal intensity after injection was observed within 15 days of treatment, and signals remained abnormal for the following 75 days.

More recently, transport into human discs has been studied. For practical reasons, most of these investigations have been limited to short time intervals between injection and imaging, hence contrast media only penetrated into the outer rim of the disc.[17,39,40] Nevertheless, studies on 19 human discs found that contrast enhancement in the endplate regions was significantly lower in discs that showed some degenerative changes,[17] indicating that transport into these discs was impaired. A separate study found that surgical treatment enhanced transport significantly.[40] Further studies using sophisticated imaging protocols[41] and mathematical modeling showed transport into the human discs studies was governed primarily by diffusion.

A comprehensive recent study[42] has investigated changes in intensity in discs for up to 24 hours following injection of gadodiamide; the authors investigated discs of 10 volunteers with no back problems and of 43 patients (in total 96 normal and 54 degenerate discs). Three different regions were examined at each time point via the vertebral body, endplate region, and disc center. Penetration into discs was slow, with peak intensities in the

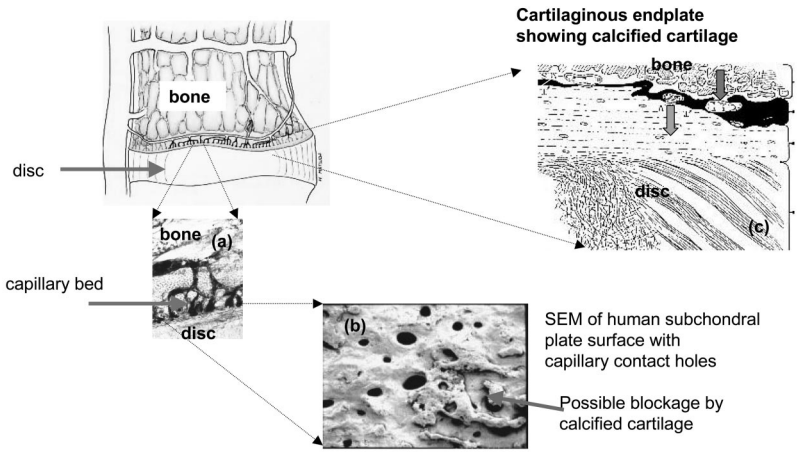

Figure 2. Schematic view of the blood supply from the vertebral body to the disc showing (**a**) details of the capillary bed at the subchondral bone/nucleus junction (adapted from Crock and Goldwasser[56]); (**b**) "holes" through the subchondral plate allowing capillary penetration with possible evidence of partial blockage by calcified cartilage (adapted from Ayotte *et al*[92]); and (**c**) schematic section through the endplate-disc bone junction showing the cartilaginous endplate and calcified cartilage (adapted from Roberts *et al*[69]).

nucleus reached after 6 hours and the signal persisting the central disc for at least 24 hours. Age had a significant effect, with intensities the greatest in young (<10 years) spines. In agreement with an earlier study,[17] signal intensity was lower in slightly degenerate than normal discs. As the degeneration grade increased, changes in signal intensity were variable, increasing in some discs, whereas marked irregularities were found in the endplate region in particular.

In these and other studies,[41,43] MRI enhancement studies have now been shown to be a powerful tool for studying transport into the disc. However, interpretation of results still requires some development. The contrast media are relatively large molecules (≥900 MW), thus signal intensity is governed by both permeability of the endplate-subchondral bone region and by the partition coefficient; it is not apparent how these two effects can be disentangled. Because the partition coefficient of gadolinium in a disc that is somewhat degenerate and has lost glycosaminoglycans will be greater than that in a normal disc,[36] a high signal in a disc could result from faster transport into it because the endplate permeability was high or, alternatively, it could indicate the disc was degenerate and thus allowed greater penetration of contrast medium. Further development of MRI protocols and application of theoretical models might allow disentanglement of these effects.

Electrode Studies

Although MRI methods follow the movement of larger solutes into the disc, measurement of transport using microelectrodes has been developed to monitor movement of dissolved gases into the disc.[44] Such electrodes have been used to monitor changes in oxygen concentrations in dog discs following an increase in blood oxygen; O_2 tension in the disc increased only slowly, in line with the diffusion theory.[45] Electrodes have also been used to determine H_2 washout from discs after closing off the different transport routes; these experiments showed that most of the disc depended on transport from the endplate, and only the outer sections of the anulus obtained their nutrients from the blood vessels at the anulus periphery.[46] Nitrous oxide, administered as an anesthetic gas and monitored using microelectrodes, can also be used as a tracer for determining transport of dissolved gases; because it is small and freely soluble, unlike MRI contrast media, it is not excluded from any area of the disc and as it is not metabolized, its concentration reflects transport considerations only. Nitrous oxide measurements have shown that transport into scoliotic discs is severely impeded particularly at the curve apex[47] and at the convexity possibly because endplate calcification acts as a barrier to nutrient transport (Figure 2).

■ What Governs Nutrient Transport Into the Disc?

The *in vivo* studies described above have been able to show some important features governing nutrient transport into the disc. They have demonstrated that there are two distinct routes into the disc via the endplate and the anulus periphery (Figure 1) and that most of the disc relies on nutrients supplied by the endplate route. They have shown that changes to the blood supply or to the endplate itself can have a marked effect on the supply of solutes to the disc. These studies have also indicated the importance of solute size and charge on transport through the disc matrix and that solutes move through the disc mainly by diffusion. Most important, they have also been able to demonstrate that nutrient supply to the disc is affected by disc pathology. These factors will be discussed in more detail below.

Blood Supply to the Disc

There is little information on the nutrient supply to the disc via the anulus periphery. However, this area of the disc appears well vascularized, more so in children than in adults.[49] In infants, blood vessels penetrate into the anulus but these disappear by late childhood apart from some small capillaries, accompanied by lymph vessels, in the outer 1 to 2 mm of the disc.[48,49]

The blood supply through the endplate appears more at risk and is better described (Figure 2). The arterial and venous systems feeding the vertebral bodies have been well described by Crock *et al* and others.[50–53] The outer region of the vertebral body, the midanulus region, and

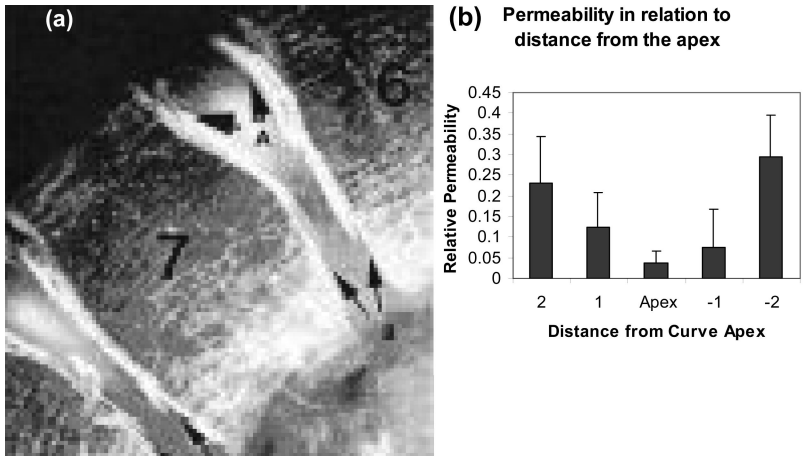

Figure 3. Effect of calcified layer on transport into scoliotic discs showing (**a**) dense layer of calcified cartilage in a scoliotic spine (arrows); and (**b**) relative nutrient transport into scoliotic discs depends on distance from the apex with transport into the apical disc most restricted (from Urban *et al*[48])

the central core of the vertebral body are each supplied by different arteries; blood flow, measured by microspheres in pigs, is highest into the cervical and lowest into the lumbar vertebrae.[54] The disc itself is supplied by capillaries that are fed from these arteries and drain into the subchondral venous network or into the veins of the marrow spaces of the vertebral bodies.[55] These capillaries have muscuranic receptors[11] that regulate blood flow in response to external signals, possibly explaining loss of nutrient transport in response to smoking[10] and vibration.[9]

The capillaries penetrate channels in the subchondral plate and terminate in loops at the bone–cartilage junction[55,56] (Figure 2). In the fetus and in infants, the subchondral plate is also penetrated by regularly spaced nutrient canals similar to those seen in other growth cartilages,[57] but these disappear in childhood, leaving residual "weak spots" that may later lead to Schmorl nodes and even later, to sclerosis of the subchondral plate.[58] The density of the capillary bed is greatest in the central region of the disc and diminishes towards the outer anulus to disappear in the region of the apophyseal ring. The density and integrity of these capillary beds diminish with age[59,60] and vary between species; the area available for nutrient exchange was estimated to be about 70% in greyhound dogs but only approximately 36% in adult humans.[61,62] Injuries to the disc,[63] sclerosis of the subchondral plate,[64] and mechanical environment[27] are all reported to affect the architecture of the capillary bed or the porosity of the subchondral plate with important consequences for delivery of nutrients to the disc.

Cartilaginous Endplate

Nutrients supplied by the capillaries and nutrient canals have to penetrate a dense hyaline cartilage endplate before reaching the disc matrix. The composition of this endplate in human adults is similar to that of other hyaline cartilages, but it is less hydrated than hip or knee cartilage.[65,66] Like other cartilages, the endplate acts as a selectively permeable barrier to solutes with both fall in hydration and increase in proteoglycan concentration restricting solute transport. Small, uncharged solutes (oxygen, amino acids, water) diffused across the endplate readily, but anions were partially excluded and the extent of steric exclusion of larger solutes increased with molecular weight and was greater for linear than globular molecules.[14]

Calcification of the endplate can act as a significant barrier to nutrient transport. The cartilaginous endplate thickness diminishes with age and calcifies by unknown mechanisms[59]; intense calcification has also been observed in scoliotic discs.[67] It has been shown both *in vitro* and *in vivo* that calcification found in scoliotic discs can impede transport of even small molecules[14,47] (Figure 3). *In vitro* tests have also shown that transport of dyes into the disc across the endplate-subchondral bone was much reduced in degenerate discs and in many cases, solutes encountered a direct barrier to further penetration possibly arising because of subchondral sclerosis or endplate calcification.[2] It is clear that calcification will restrict nutrient supply to the disc cells. Whether calcification causes disc degeneration because disc cells die or change metabolism, or alternatively whether degenerative changes cause alterations in the physical and mechanical environment of endplate cells leading to calcification is still not apparent.

Transport Through the Matrix

Transport through the matrix is governed by both properties of the matrix and of the solute. The matrix consists mainly of a collagen network embedded in a dense polyanionic proteoglycan gel, which acts as a selective permeability barrier to entry of molecules into the disc. Only low concentrations of large molecules (*e.g.*, growth factors, protease inhibitors) can enter the disc; even glucose (MW180) is restricted to some extent.[29] Charge effects, however, enhance the penetration of small cations such as sodium in direct proportion to the proteoglycan concentration, whereas anions such as sulfate and chloride are partially excluded.[29] Because the proteoglycan concentration is higher in the nucleus than the outer anulus, large solutes and negatively charged molecules can enter into the anulus more readily than into the nucleus. The rate at which molecules can diffuse through the matrix is

also affected by the composition of the matrix, particularly by the concentration of proteoglycans. In general, the diffusion coefficient of nutrients is only 40% to 50% of that in free aqueous solutions; experimental work in other tissues has shown that if proteoglycans are removed enzymatically, solute diffusivity rises towards that in external solutions[68]

Convection or Diffusion. Several tracer and MRI studies support the view that nutrients move into the disc under concentration gradients, which arise as the result of cellular metabolism. Nevertheless, in view of the diurnal loading cycle of the disc with consequent loss and regain of around 25% of the disc's fluid,[69] it has long been suggested that fluid movement is necessary for transport of nutrients into the disc. Against this view is the finding that for small solutes such as the sulfate ion and oxygen, there is good correspondence between transport gradients predicted by diffusion theory and those measured experimentally both for anesthetized and exercised animals.[26,62,70] Theoretical and experimental considerations support the finding that small molecules can move through the matrix faster by diffusion than by convection.[71–73] It thus seems that the cells rely on diffusion rather than convection for their supply of essential nutrients such as oxygen and glucose. Indeed, if convective transport of nutrients was faster than diffusion, because convective transport is outward rather than inward during the day's activities, the cells could be deprived of essential nutrients for a large part of the day. For large solutes, however, such as growth factors, proteases, and their inhibitors, which have a much lower diffusivity,[74] convective movement may contribute significantly to their movement through the matrix. The role of convection may also be important in governing movement of newly synthesized matrix macromolecules through the matrix and in determining the rate of loss of matrix breakdown products.

The diurnal cycle of load-induced fluid expression and regain, however, could have important consequences for transport because factors affecting diffusion, such as solute diffusivities, partitions,[74,75] and disc height (diffusion distance), are sensitive to hydration. Changes in loading arising could also affect capillary blood flow, but at present, this area is unexplored. Thus, fluid movement in and out of the disc and the consequent changes in matrix properties could affect nutrient transport, even if not directly by entrainment; at present, its effects are unclear.

Cell Metabolism

Disc cells require nutrients to stay alive and to function. Their main supply of energy is provided by glycolysis[4,76,77]; thus, they consume glucose and produce lactic acid at a relatively high rate. An adequate level of glucose is essential for maintaining the viability of disc cells, and if the glucose concentration drops below around 0.5 mmol/L for more than a few days, the cells begin to die[78,79] (Figure 4). Low pH (<6.4) resulting primarily from accumulation of lactic acid, also compromises disc

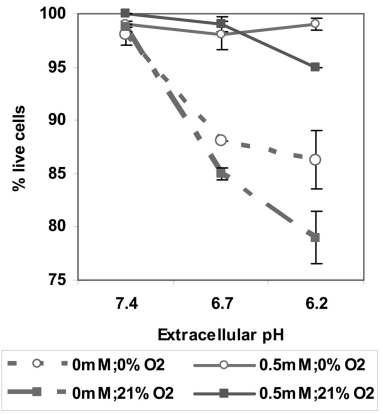

Figure 4. Effect of nutrient deprivation on death of disc nucleus cells measured in culture *in vitro*. The figure shows a significant loss of viability with 24 hours of culture in cells deprived of glucose and at low pH both in the presence and absence of oxygen, whereas with glucose present, cells survive even if pH is acidic and oxygen is removed.[93]

cell viability[78,79] (Figure 4). Less acidic conditions, although not necessarily leading to cell death, are also detrimental to disc matrix integrity, as they reduce the rate at which the cells synthesize matrix components but not the rate at which they make active proteases, *i.e.*, agents able to break down the matrix.[80] Disc cells also consume oxygen at a substantial rate but produce relatively little CO_2,[77] so it is not apparent how much of their energy is provided by oxidative phosphorylation. They can survive many days (at least up to 14 days) with no oxygen present[78,79]; under hypoxia, however, they are inactive and matrix synthesis is severely reduced,[76,78] although in some studies, glycolysis appears stimulated (a positive Pasteur effect).[4,76,77] The role of oxygen in disc metabolism, as in articular cartilage,[81] is thus unclear.

Because disc cells use glucose to produce adenosine triphosphate (ATP), with lactic acid as the resulting metabolite, the concentrations of glucose and lactic acid are necessarily coupled as are oxygen and lactic acid concentrations through the Pasteur effect.[4,76] The center of the disc thus experiences low oxygen concentrations together with low glucose and high lactic acid concentrations[4] (and hence acidic levels of pH[16]) (Figure 5).

This microenvironment, as well as affecting matrix synthesis, also affects rates of energy metabolism. Both oxygen consumption and lactate production rates are strongly affected by both oxygen concentration and pH particularly under acidic conditions and hypoxia.[79] These rates of metabolism have a strong effect on the local nutrient environment because nutrients are supplied to the disc cells by diffusion under gradients set up by cellular metabolism. The steepness of the gradient, and thus the concentration of nutrient available to the cell at any point in the disc, depends on the balance between the rate at which the solute can move through the tissue by diffusion and the rate at which it is consumed. For example, the sulfate ion and glucose have similar molecular weights and diffusivities, so they are

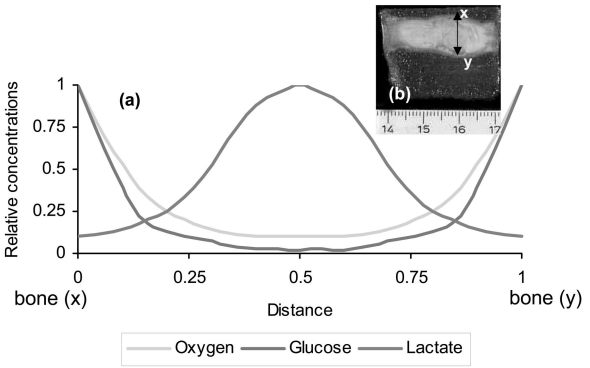

Figure 5. Schematic view of nutrient gradients across the disc nucleus endplate-endplate. **a,** oxygen and glucose concentrations fall and lactic acid concentrations rise towards the center of the nucleus; hence, the center of the disc is at low glucose and oxygen and is acidic. **b,** a sagittal section through a human lumbar disc showing the dimensions of the disc and the direction of the gradient shown in **a**.

able to diffuse through the matrix at similar rates. In the disc, the rate of consumption per volume of tissue of glucose, the main source of cellular energy, is 10 to 100 times the rate of incorporation of sulfate, an essential component of proteoglycans; the estimated gradient of sulfate is hence flat because sulfate can be replenished faster that it is used, whereas the concentration gradient of glucose (and of lactic acid) is steep.[61]

Diffusional gradients are also directly regulated by cell density, because the higher the cell number, the higher the cellular demand. Conversely, cell density appears to depend on nutrient supply. *In situ*, the cell density throughout the disc is not uniform but is highest at the anulus edge, which is closest to the nutrient supply and then falls steeply with distance (Figure 6a).[61] Average cell density through the nucleus is highest in small animals where diffusional distances are small and falls exponentially with increase in disc height[82] (Figure 6b), as seen also in other avascular cartilages.[83] A similar inverse relationship between viable cell density and diffusional distance has been observed in cell culture *in vitro*. The cell density in the disc thus appears to be regulated by nutritional constraints *i.e.*, the number of viable cells that can be supported depends on the nutrient supply. Loss of nutrient supply could thus lead to cell death, as is seen in scoliotic discs, where endplate calcification[14,47] (Figure 3) appears to lead to cell death.[18,19]

■ Modeling the Nutrient Supply

Because of lack of appropriate animal models and because of the difficulties in measurements in humans, mathematical models of nutrient transport may give an insight into the overall consequence of disturbances to blood supply or endplate calcification that cannot be acquired by other means. Because nutritional factors are thought to be such an important factor in development of disc degeneration, and yet experiments are difficult and time consuming, it is surprising that there are so few studies of modeling of transport. The few modeling studies so far carried out show the potential of such models for elucidating how disturbances to nutrient supply or to disc properties could influence the extracellular nutrient microenvironment and hence affect cellular metabolism and viability.

For an accurate, all-encompassing model of disc nutrition, some of the factors to be considered are: 1) the disc's avascular nature and the architecture and efficiency of the peripheral vascular system; 2) its size and shape; 3) its hydration and matrix composition; 4) its transport parameters (such as hydraulic permeability) and their hydration dependence; 5) the properties of the solute of interest (e.g. binding, charge, diffusivity in the disc); 6) the permeability of the cartilaginous endplate; 7) distribution of cell phenotype and cell density; 8) cellular metabolic activity; 9) the biomechanical loading effects on disc shape, hydration, transport and cellular activity. No model has yet undertaken to incorporate all these variable and indeed, the quantitative data necessary for building such a model are not available. However, the simpler models developed over the past few decades have provided some understanding of factors important in regulating nutrient supply to the disc.

The earliest nutrient transport models were simple one-dimensional analytical models that regarded cellular consumption rate as a constant and only considered nutrient transport by diffusion. These calculations indicated that the glucose supply to the central region of the disc was precarious[61] and that concentrations of both oxygen and glucose in the center of the disc were low and

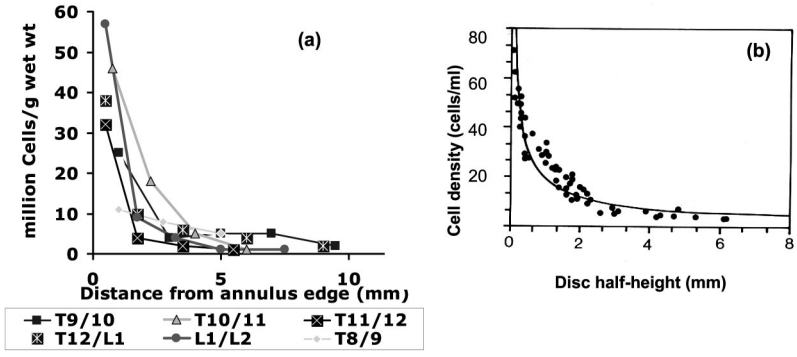

Figure 6. Cell density in the disc: (**a**) gradients in cell density from the outer anulus towards the disc center in 6 discs removed at surgery from a 12-year-old human scoliotic spine[19]; (**b**) average cell density in the nucleus *versus* disc height measured in a variety of different animal species.[87]

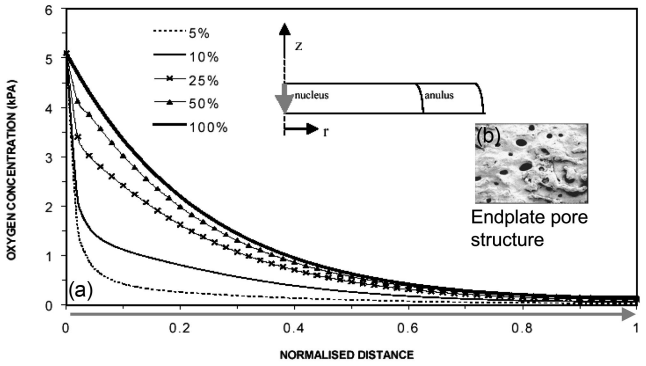

Figure 7. Variation of oxygen concentration through the disc with variation in exchange area at the vertebral body–disc junction. **a,** effect of fall in exchange area on oxygen concentration profiles from the endplate to the center of the nucleus (direction shown by green arrow)(adapted from Selard *et al*[88]). **b,** human subchondral bone surface exchange area (from Figure 2), *i.e.,* fraction of total area containing holes for capillary penetration.

of lactic acid were high in agreement with experimental measurements. They also showed that experimental measurements of oxygen gradients[70] or of transport of small solutes into the disc could be explained satisfactorily by diffusion alone[62] even under conditions of fluid movement.[70,84]

These calculations did not investigate how changes in nutrient delivery or in properties of the disc influenced nutrient gradients. These problems were first explored by Stairmand *et al*,[85] who also considered the effects of a concentration-dependent consumption rate using Michaelis-Menton kinetics to describe oxygen consumption rate and the published relationship between disc cell density and disc thickness.[82] The model was solved numerically by a finite difference method. Oxygen gradients compared well with data from dog disc experiments,[4] and calculation showed how the nature of the oxygen consumption term (*i.e.*, fall in consumption rate with fall in concentration) protected the central area from complete oxygen depletion. This study highlighted the importance of accurate exchange area, cell density, and disc thickness incorporation into models.

Selard *et al*[86] extended this model by considering diffusive transport into the disc from both endplate and anulus periphery with metabolic consumption of oxygen and glucose and production of lactate. This diffusion-reaction model showed that finite element models (FEM) could be used for modeling disc nutrient transport. The effects of endplate permeability (Figure 7), disc height, and changes in metabolic rates and diffusivities were investigated. Results showed that concentration gradients of oxygen, glucose, and lactate (Figure 8) were all sensitive to consumption rate and diffusivity as well as factors more often considered as critical regulators of transport, such as delivery of nutrients through the endplate (Figure 7).

The effect of fluid exchange and convective transport were recently investigated by Ferguson *et al*.[73] The model considered transport of a range of solutes based on reported solute diffusivity, but did not alter diffusion coefficient with hydration fraction. The results show that convective enhancement is generally insignificant for molecules of size <1 kDa (oxygen, glucose, and lactate), but extremely important for larger species such as TIMP-1 (28 kDa), cytokines (10–40 kDa), and proteoglycans (>40 kDa). This theoretical size distinction thus supported the assumption that convection was negligible for transport of oxygen, glucose, and lactate and validates results of earlier models of nutrient transport.

In summary, gradients of oxygen, glucose, and lactic acid exist throughout the disc (Figure 5), and the local concentrations (particularly towards the center of the disc, where they will be most extreme) have a strong influence on metabolic rates, matrix production, and cell survival[76,79,80] and hence on the development of disc degeneration. Rates of metabolism in conjunction with variables such as endplate permeability,[47] diffusion coefficients,[74]cell density,[19,61] and load-induced strains, pressure rises, and fluid movement can be used to predict profiles of nutrients and metabolites in the intervertebral disc. Such models could lead to a better understanding of the conditions occurring in the center of the avascular disc and enable predictions to be made about the metabolic state of the disc *in vivo* and conditions that promote

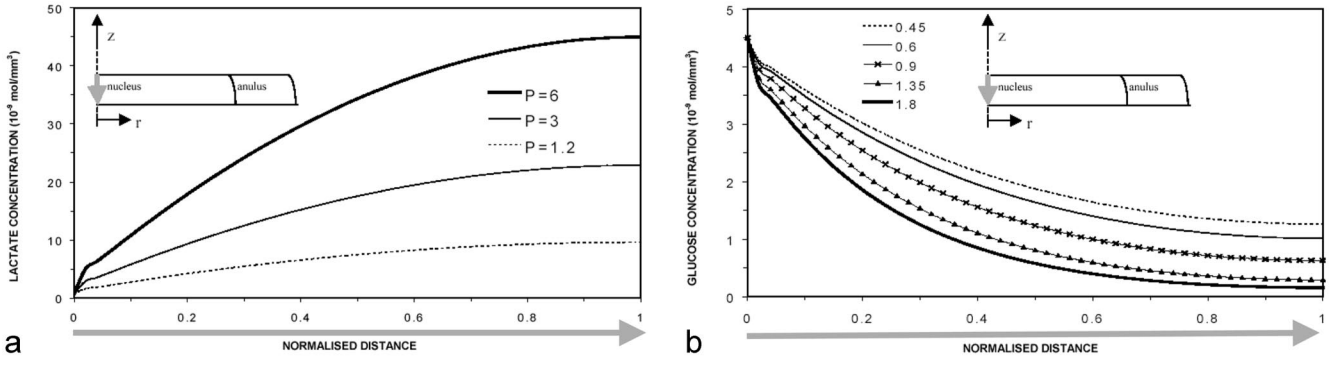

Figure 8. Effect of cellular activity on concentration gradients from the endplate to the disc center; direction shown by arrows (adapted from Selard *et al*[88]). **a,** effect of lactate production rate on lactate gradients; lactate concentrations in the center increase with increase in production rate. **b,** effect of glucose consumption rate on glucose gradients; glucose concentrations in the center fall with increase in glucose consumption rate.

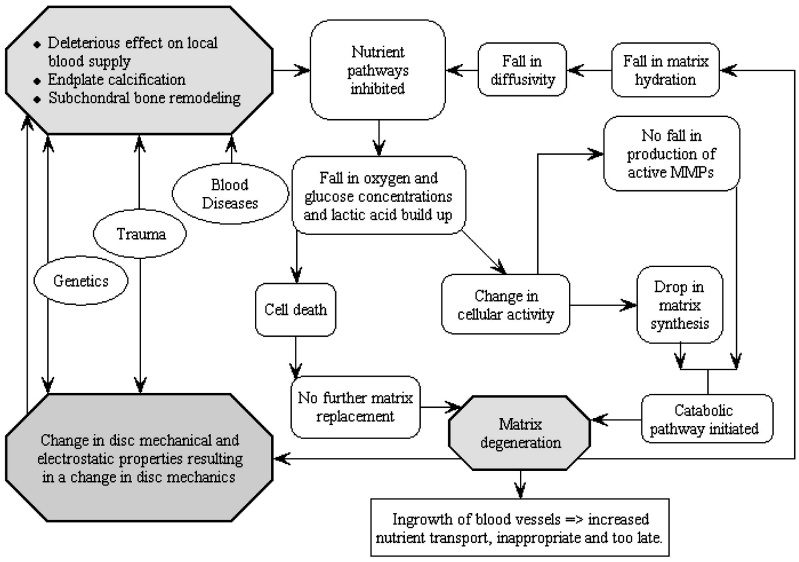

Figure 9. Relationship between loss of nutrient supply and disc degeneration: a flow chart showing some possible pathways.

or disturb cell survival and disc integrity. However, these models only have predictive capability if they are based on good data, particularly in regard to cellular requirements and to cellular responses to environmental signals such as growth factors, nutrient concentrations, and loading. At present, there are few quantitative data in this regard.

■ Consequences of Loss of Nutrient Supply

Both modeling and experimental work have demonstrated the existence of steep gradients in oxygen concentration and pH throughout the disc (Figures 5, 7, and 8), so that concentrations of nutrients in the center of the disc are much lower than those at the disc center. The actual level to which oxygen and pH fall can have a strong influence on matrix turnover. Rates of sulfated glycosaminoglycan production appear to maximize at around pH 7.0 and at 5% to 10% oxygen[76,87] rather than under the culture conditions commonly used *in vitro* (21% oxygen, pH 7.4). The reasons for the fall in matrix synthesis at higher oxygen and pH levels is unclear; perhaps because disc cells normally are not exposed to high oxygen, they do not have adequate mechanisms for removing the reactive oxygen species produced at 21% oxygen. Such results do indicate, however, the importance of studying the behavior of disc cells under the physical and chemical conditions encountered *in* situ.[88]

If, however, the nutrition of the disc is impaired so that nutrient concentrations fall to critical levels (possibly below 0.5 mmol/L glucose and pH6.3), disc cells will die (Figure 6); acidic pH levels in this range have been seen in degenerate and symptomatic discs.[15,16] Moreover, even if nutrient concentrations do not fall below critical values, low oxygen and acidic pH levels can have adverse effects on matrix synthesis. In cell culture, matrix synthesis rates fall rapidly once oxygen concentrations fall below 5% oxygen[76] or pH falls below 6.8.[80] Moreover, under acidic conditions, disc cells continue to produce active matrix metalloproteinases, *i.e.*, agents able to break down the macromolecular constituents of the disc matrix[80] even though matrix production is inhibited. Low pH could thus rapidly lead the disc down a destructive pathway (Figure 9) even if the cells remain viable.

■ Disc Repair and Disc Nutrition

There is now considerable interest in the possibility of inducing disc repair by stimulating the native cell population to greater activity by supplying exogenous growth factors (reviewed by An *et al* in this issue of *Spine*) or through gene therapy (reviewed by Kang *et al* in this issue). Approaches based on the success of autologous cartilage implantation[89] are also being vigorously pursued; here, repair of the matrix is induced by inserting a disc or differentiated stem cells into the damaged or degenerate disc; a clinical study using this approach is underway.[90] Even tissue-engineering approaches, where disc cells are introduced into scaffolds and cultured *in vitro* to produce disc matrix suitable for disc repair,[91] are under investigation. These approaches all seem to be successful in animal studies where these maneuvers have been found to restore disc height and matrix growth to some extent. It should be noted, however, that the animal models of disc degeneration are all of relatively short duration, induced in young and previously healthy animal discs where the effects of the induced degeneration on disc nutrition are unknown, but in some cases at least, these do not appear to be substantial.[63]

New methods of disc repair involving stimulation of native cells or insertion of new cells or tissue-engineered disc should, however, be used with caution in humans. For successful disc repair, the newly inserted or stimulated cells have to exist in conditions where they remain viable and active. It is thus essential that the nutrient supply to the disc is adequate and, moreover, that it can support the increased nutritional demands these meth-

ods induce; the disc will need more nutrients to support inserted cells or cells for which the activity has been increased by supplying growth factors. Because failure of nutrient supply is thought to be a major cause of disc degeneration (Figure 9) and a high proportion of degenerate discs do indeed appear to have an impaired nutrient supply,[2,42] it is unrealistic to expect that reimplantation of cells into such discs would effect a repair. Before cell-based therapies can be introduced successfully, some method of selecting suitable patients on the basis of an adequate nutrient supply to the affected disc appears absolutely crucial.

■ Key Points

- The disc is avascular.
- Essential nutrients are supplied to the disc virtually entirely by diffusion.
- Cellular activity and viability depends on the maintenance of adequate pericellular concentrations of glucose and oxygen and on regulation of pH at acceptable levels.
- Levels of nutrient throughout the disc are governed by the balance between factors regulating supply of nutrients and those regulating cellular demand.
- A critical area for nutrient supply appears to be the subchondral bone–cartilage endplate–disc junction, which can become blocked, causing nutrient concentrations throughout the disc to fall to critically low levels.
- Loss of nutrient supply is likely to lead to matrix degradation and to cell death and hence to disc degeneration.

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References

1. Oegema TR. Biochemistry of the intervertebral disc. *Clin Sports Med* 1993; 12:419–39.
2. Nachemson A, Lewin T, Maroudas A, et al. In vitro diffusion of dye through the endplates and annulus fibrosus of human lumbar intervertebral discs. *Acta Orthop Scand* 1970;41:589–607.
3. Eyre DR, Caterson B, Benya P, et al. The intervertebral disc. In: Gordon S, Frymoyer J, eds. *New Perspectives on Low Back Pain*. Philadelphia, PA: American Institute of Orthopaedic Surgeons; 1991:147–209.
4. Holm S, Maroudas A, Urban JP, et al. Nutrition of the intervertebral disc: solute transport and metabolism. *Connect Tissue Res* 1981;8:101–19.
5. Kurunlahti M, Tervonen O, Vanharanta H, et al. Association of atherosclerosis with low back pain and the degree of disc degeneration. *Spine* 1999; 24:2080–4.
6. Kauppila LI. Prevalence of stenotic changes in arteries supplying the lumbar spine. A postmortem angiographic study on 140 subjects. *Ann Rheum Dis* 1997;56:591–5.
7. Babhulkar S, Babhulkar S. Osteonecrosis in sickle cell hemoglobinopathy. In: Urbaniak JR, Jones JPJ, eds. *Osteonecrosis—Etiology, Diagnosis and Treatment*. American Orthopaedic Association; 1997:131–3.
8. Jones JPJ. Subchondral osteonecrosis can conceivably cause disk degeneration and primary osteoarthritis. In: Urbaniak JR, Jones JPJ, eds. *Osteonecrosis—Etiology, Diagnosis and Treatment*. American Orthopaedic Association; 1997:135–42.
9. Hirano N, Tsuji H, Ohshima H, et al. Analysis of rabbit intervertebral disc physiology based on water metabolism: II. changes in normal intervertebral discs under axial vibratory load. *Spine* 1988;13:1297–302.
10. Holm S, Nachemson A. Nutrition of the intervertebral disc: acute effects of cigarette smoking. An experimental animal study. *Ups J Med Sci* 1988;93: 91–9.
11. Wallace AL, Wyatt BC, McCarthy ID, et al. Humoral regulation of blood flow in the vertebral endplate. *Spine* 1994;19:1324–8.
12. Deyo RA, Bass JE. Lifestyle and low back pain: the influence of smoking and obesity. *Spine* 1989;14:501–6.
13. Wilder DG, Pope MH. Epidemiological and etiological aspects of low back pain in vibration environments. *Clin Biomech (Bristol, Avon)* 1996;11:61–73.
14. Roberts S, Urban JPG, Evans H, et al. Transport properties of the human cartilage endplate in relation to its composition and calcification. *Spine* 1996;21:415–20.
15. Kitano T, Zerwekh JE, Usui Y, et al. Biochemical changes associated with the symptomatic human intervertebral disk. *Clin Orthop* 1993;372–7.
16. Diamant B, Karlsson J, Nachemson A. Correlation between lactate levels and pH in discs of patients with lumbar rhizopathies. *Experientia* 1968;24: 1195–6.
17. Nguyen-Minh C, Haughton VM, Papke RA, et al. Measuring diffusion of solutes into intervertebral disks with MR imaging and paramagnetic contrast medium. *AJNR Am J Neuroradiol* 1998;19:1781–4.
18. Bibby SR, Fairbank JC, Urban MR, et al. Cell viability in scoliotic discs in relation to disc deformity and nutrient levels. *Spine* 2002;27:2220–8.
19. Urban MR, Fairbank JC, Bibby SR, et al. Intervertebral disc composition in neuromuscular scoliosis: changes in cell density and glycosaminoglycan concentration at the curve apex. *Spine* 2001;26:610–7.
20. Brodin H. Paths of nutrition in articular cartilage and intervertebral discs. *Acta Orthop Scand* 1955;24:177–83.
21. Brown MD, Tsaltas T. Studies on the permeability of the intervertebral disc during skeletal maturation. *Spine* 1976;1:240–4.
22. Kramer J, Kroner H. [Autoradiographic investigations of transverse sections of intervertebral discs (author's translation)]. *Z Orthop Ihre Grenzgeb* 1973; 111:147–9.
23. Adams MA, Hutton WC. The effect of posture on diffusion into lumbar intervertebral discs. *J Anat* 1986;147:121–34.
24. Ohshima H, Tsuji H, Hirano N, et al. Water diffusion pathway, swelling presure and biomechanical properties of the intervertebral disc during compression loading. *Spine* 1991;14:1234–44.
25. Terahata N, Ishihara H, Ohshima H, et al. Effects of axial traction stress on solute transport and proteoglycan synthesis in the porcine intervertebral disc in vitro. *Eur Spine J* 1994;3:325–30.
26. Urban JP, Holm S, Maroudas A, et al. Nutrition of the intervertebral disc: effect of fluid flow on solute transport. *Clin Orthop* 1982;296–302.
27. Holm S, Nachemson A. Variation in the nutrition of the canine intervertebral disc induced by motion. *Spine* 1983;8:866–74.
28. Holm S, Nachemson A. Nutritional changes in the canine intervertebral disc after spinal fusion. *Clin Orthop* 1982;169:243–58.
29. Urban JPG, Maroudas A. Measurement of fixed charge density and partition coefficients in the intervertebral disc. *Biochim Biophys Acta* 1979;586:166–78.
30. Shibuya K. Experimental and clinical studies on metabolism with the intervertebral disc. *J Japan Orthop Assoc* 1970;44:1–24.
31. Thomas RW, Batten JJ, Want S, et al. A new in-vitro model to investigate antibiotic penetration of the intervertebral disc. *J Bone Joint Surg Br* 1995; 77:967–70.
32. Riley LH III, Banovac K, Martinez OV, et al. Tissue distribution of antibiotics in the intervertebral disc. *Spine* 1994;19:2619–25.
33. Tai CC, Want S, Quraishi NA, et al. Antibiotic prophylaxis in surgery of the intervertebral disc. A comparison between gentamicin and cefuroxime. *J Bone Joint Surg Br* 2002;84:1036–9.
34. Ibrahim MA, Jesmanowicz A, Hyde JS, et al. Contrast enhancement of normal intervertebral disks: time and dose dependence. *AJNR Am J Neuroradiol* 1994;15:419–23.
35. Ibrahim MA, Haughton VM, Hyde JS. Effect of disk maturation on diffusion of low-molecular-weight gadolinium complexes: an experimental study in rabbits. *AJNR Am J Neuroradiol* 1995;16:1307–11.
36. Bashir A, Gray ML, Hartke J, et al. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. *Magn Reson Med* 1999;41: 857–65.
37. Perlewitz TJ, Haughton VM, Riley LH III, et al. Effect of molecular weight on the diffusion of contrast media into cartilage. *Spine* 1997;22:2707–10.
38. Nguyenminh C, Riley L, Ho KC, et al. Effect of degeneration of the intervertebral disk on the process of diffusion. *AJNR Am J Neuroradiol* 1997;18: 435–42.

39. Akansel G, Haughton VM, Papke RA, et al. Diffusion into human intervertebral disks studied with MR and gadoteridol. *AJNR Am J Neuroradiol* 1997;18:443–5.
40. Ross JS, Zepp R, Modic MT. The postoperative lumbar spine: enhanced MR evaluation of the intervertebral disk. *AJNR Am J Neuroradiol* 1996;17:323–31.
41. Bydder GM. New approaches to magnetic resonance imaging of intervertebral discs, tendons, ligaments, and menisci. *Spine* 2002;27:1264–8.
42. Rajasekharan S. A study of diffusion into the intervertebral disc. *Spine* 2004. In press.
43. Young IR, Bydder GM. Magnetic resonance: new approaches to imaging of the musculoskeletal system. *Physiol Meas* 2003;24:R1–23.
44. O'Hare D, Winlove CP, Parker KH. Electrochemical method for direct measurement of oxygen concentration and diffusivity in the intervertebral disc: electrochemical characterization and tissue-sensor interactions. *J Biomed Eng* 1991;13:304–12.
45. Holm S, Selstam G. Oxygen tension alterations in the intervertebral disc as a response to changes in the arterial blood. *Ups J Med Sci* 1982;87:163–74.
46. Ogata K. 1980 Volvo award winner in basic science. Nutritional pathways of the intervertebral disc. An experimental study using hydrogen washout technique. *Spine* 1981;6:211–6.
47. Urban MR, Fairbank JC, Etherington PJ, et al. Electrochemical measurement of transport into scoliotic intervertebral discs in vivo using nitrous oxide as a tracer. *Spine* 2001;26:984–90.
48. Hassler O. The human intervertebral disc. A micro-angiographical study of its vascular supply at various ages. *Acta Orthop Scand* 1970;40:765–72.
49. Rudert M, Tillmann B. Detection of lymph and blood vessels in the human intervertebral disc by histochemical and immunohistochemical methods. *Ann Anat* 1993;175:237–42.
50. Crock HV, Goldwasser M, Yoshizawa H. Vascular anatomy related to the intervertebral disc. In: Ghosh P, ed. *Biology of the Intervertebral Disc*. Boca Raton, FL: CRC Press; 1991:109–33.
51. Ratcliffe JF. The arterial anatomy of the adult human lumbar vertebral body: a microarteriographic study. *J Anat* 1980;131:57–79.
52. Crock HV, Yoshizawa H, Kame SK. Observations on the venous drainage of the human vertebral body. *J Bone Joint Surg Br* 1973;55:528–33.
53. Yoshizawa H, Ohiwa T, Kubota K, et al. Morphological study on the vertebral route for the nutrition of the intervertebral disc. *Neuro-Orthopedics* 1986;1:17–32.
54. Drescher W, Li H, Qvesel D, et al. Vertebral blood flow and bone mineral density during long-term corticosteroid treatment: an experimental study in immature pigs. *Spine* 2000;25:3021–5.
55. Crock HV, Goldwasser M. Anatomic studies of the circulation in the region of the verterbal end-plate in adult greyhound dogs. *Spine* 1984;9:702–6.
56. Oki S, Matsuda Y, Shibata T, et al. Morphologic differences of the vascular buds in the vertebral endplate: scanning electron microscopic study. *Spine* 1996;21:174–7.
57. Whalen JL, Parke WW, Mazur JM, et al. The intrinsic vasculature of developing vertebral end plates and its nutritive significance to the intervertebral discs. *J Pediatr Orthop* 1985;5:403–10.
58. Chandraraj S, Briggs CA, Opeskin K. Disc herniations in the young and end-plate vascularity. *Clin Anat* 1998;11:171–6.
59. Bernick S, Cailliet R. Vertebral end-plate changes with aging of human vertebrae. *Spine* 1982;7:97–102.
60. Donisch EW, Trapp W. The cartilage endplates of the human vertebral column (some considerations of postnatal development). *Anat Rec* 1971; 169:705–16.
61. Maroudas A, Nachemson A, Stockwell R, et al. Factors involved in the nutrition of the human lumbar intervertebral disc: cellularity and diffusion of glucose in vitro. *J Anat* 1975;120:113–30.
62. Urban JP, Holm S, Maroudas A. Diffusion of small solutes into the intervertebral disc: as in vivo study. *Biorheology* 1978;15:203–21.
63. Moore RJ, Osti OL, Vernon-Roberts B, et al. Changes in endplate vascularity after an outer anulus tear in the sheep. *Spine* 1992;17:874–8.
64. Ayotte DC, Ito K, Perren SM, et al. Direction-dependent constriction flow in a poroelastic solid: the intervertebral disc valve. *J Biomech Eng* 2000;122: 587–93.
65. Roberts S, Menage J, and Urban JPG. Biochemical and structural properties of the cartilage end-plate and its relation to the intervertebral disc. *Spine* 1989;14:166–74.
66. Setton LA, Zhu W, Weidenbaum M, et al. Compressive properties of the cartilaginous end-plate of the baboon lumbar spine. *J Orthop Res* 1993;11: 228–39.
67. Roberts S, Menage J, Eisenstein SM. The cartilage end-plate and intervertebral disc in scoliosis: calcification and other sequelae. *J Orthop Res* 1993; 11:747–57.
68. Boubriak OA, Urban JP, Akhtar S, et al. The effect of hydration and matrix composition on solute diffusion in rabbit sclera. *Exp Eye Res* 2000;71:503–14.
69. Boos N, Wallin A, Gbedegbegnon T, et al. Quantitative MR imaging of lumbar intervertebral disks and vertebral bodies: influence of diurnal water content variations. *Radiology* 1993;188:351–4.
70. Katz MM, Hargens AR, Garfin SR. Intervertebral disc nutrition. Diffusion versus convection. *Clin Orthop* 1986;243–5.
71. Mauck RL, Hung CT, Ateshian GA. Modeling of neutral solute transport in a dynamically loaded porous permeable gel: implications for articular cartilage biosynthesis and tissue engineering. *J Biomech Eng* 2003;125:602–14.
72. O'Hara BP, Urban JP, Maroudas A. Influence of cyclic loading on the nutrition of articular cartilage. *Ann Rheum Dis* 1990;49:536–9.
73. Ferguson SJ, Ito K, Nolte LP. Fluid flow and convective transport of solutes within the intervertebral disc. *J Biomech* 2004;37:213–21.
74. Boubriak OA, Urban JPG. Nutrient supply to the cells of the intervertebral disc: effect of diurnal hydration changes. *Trans Am Orthop Res Soc* 2003; 28:1127.
75. Nimer E, Schneiderman R, Maroudas A. Diffusion and partition of solutes in cartilage under static load. *Biophys Chem* 2003;106:125–46.
76. Ishihara H, Urban JP. Effects of low oxygen concentrations and metabolic inhibitors on proteoglycan and protein synthesis rates in the intervertebral disc. *J Orthop Res* 1999;17:829–35.
77. Holm S, Selstam G, Nachemson A. Carbohydrate metabolism and concentration profiles of solutes in the canine lumbar intervertebral disc. *Acta Physiol Scand* 1982;115:147–56.
78. Horner HA, Urban JP. 2001 Volvo Award Winner in Basic Science Studies. Effect of nutrient supply on the viability of cells from the nucleus pulposus of the intervertebral disc. *Spine* 2001;26:2543–9.
79. Bibby SR, Jones DA, Ripley RM, et al. Metabolism of the intervertebral disc: effects of low levels of oxygen, glucose and pH on rates of energy metabolism of bovine nucleus pulposus cells. *Trans Am Orthop Res Soc* 2004.
80. Razaq S, Wilkins RJ, Urban JP. The effect of extracellular pH on matrix turnover by cells of the bovine nucleus pulposus. *Eur Spine J* 2003;12:341–9.
81. Lee RB, Urban JP. Functional replacement of oxygen by other oxidants in articular cartilage. *Arthritis Rheum* 2002;46:3190–200.
82. Holm S, Nachemson A. Cellularity in the intervertebral disc and its relevance to nutrition. Presented at: ISSLS Proceedings; 1983.
83. Stockwell R. The inter-relationship of cell density and cartilage thickness in mammalian articular cartilage. *J Anat* 1971;109:411–22.
84. Urban JP, Holm S, Maroudas A, et al. Nutrition of the intervertebral disk. An in vivo study of solute transport. *Clin Orthop* 1977;101–14.
85. Stairmand J, Holm S, Urban J. Factors influencing oxygen concentration gradients in the intervertebral disc: a theoretical analysis. *Spine* 1991;16: 444–9.
86. Selard E, Shirazi-adl A, Urban JP. Finite element study of nutrient diffusion in the human intervertebral disc. *Spine* 2003;28:1945–53.
87. Ohshima H, Urban JPG. Effect of lactate concentrations and pH on matrix synthesis rates in the intervertebral disc. *Spine* 1992;17:1079–82.
88. Urban JP. The role of the physicochemical environment in determining disc cell behavior. *Biochem Soc Trans* 2002;30:858–64.
89. Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage* 2002;10: 432–63.
90. Ganey TM, Meisel HJ. A potential role for cell-based therapeutics in the treatment of intervertebral disc herniation. *Eur Spine J* 2002;11(suppl 2): S206–14.
91. Alini M, Roughley PJ, Antoniou J, et al. A biological approach to treating disc degeneration: not for today, but maybe for tomorrow. *Eur Spine J* 2002;11(suppl 2):S215–20.
92. Ayotte DC, Ito K, Tepic S. Direction-dependent resistance to flow in the endplate of the intervertebral disc: an ex vivo study. *J Orthop Res* 2001;19: 1073–7.
93. Bibby SR, Urban JP. Effect of nutrient deprivation on the viability of intervertebral disc cells. *Eur Spine J* 2004 Mar 27 [Epub ahead of print].