

transport, insulin receptor signaling, and response to hypoxia. Early studies suggest that GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst (Carayannopoulos et al. 2000).

For a detailed discussion of the physiological functions of GLUTs and more information about GLUT9, GLUT10, GLUT11, GLUT12, HMIT, and GLUT14, we would like to refer the readers to the AceView site (<http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/>) and a recent review by Wood and Trayhurn (Wood and Trayhurn 2003).

7 Molecular Diversity of Facilitative Glucose Transporters in Articular Chondrocytes

In order to appreciate the physiological basis for the molecular diversity of facilitative glucose transporters in chondrocytes we need to re-examine the importance of glucose as a nutrient and structural precursor. Glucose is an important nutrient in fully developed articular cartilage due to the poor vascularization and highly glycolytic nature of the tissue, a situation that is further exacerbated by low oxygen tensions and ongoing anaerobic glycolysis by chondrocytes (Mobasheri et al. 2002c; Otte 1991; Rajpurohit et al. 2002). Therefore, even modest changes in glucose concentrations in the extracellular microenvironment of chondrocytes could impair anabolic and catabolic activities (Mobasheri et al. 2002c; Shikhman et al. 2001a). Fully developed adult chondrocytes express mRNA for multiple isoforms of the GLUT/SLC2A family of glucose transporters including GLUT1, GLUT3, GLUT5, GLUT6, GLUT8, GLUT9, GLUT10, GLUT11, and GLUT12 (Mobasheri et al. 2002b, 2002c; Richardson et al. 2003; Shikhman et al. 2001a) (Fig. 8). The reason for such GLUT isoform diversity in chondrocytes has not yet been satisfactorily explained but several hypotheses have been put forward: GLUT isoform diversity in chondrocytes suggests that the transmembrane uptake of glucose, fructose, and other related hexose sugars is highly specialized and requires several proteins with the capacity to transport structurally different sugars. The observed diversity of GLUT proteins in chondrocytes may possibly reflect a cartilage-specific requirement for 'fast' (i.e., GLUT3) and baseline (GLUT1) glucose transporters that operate more efficiently at low substrate concentrations under physiological conditions (Mobasheri et al. 2002c; Richardson et al. 2003). The presence of GLUT1 in chondrocytes has also been linked to the acute requirement of these cells for glycolytic energy metabolism under the low oxygen tension conditions that are prevalent in avascular load-bearing articular cartilage and intervertebral disc (Pfander et al. 2003; Rajpurohit et al. 2002; Schipani et al. 2001). GLUT1 has also been shown to be a cytokine inducible glucose transporter in cartilage since it is induced by catabolic, proinflammatory cytokines (Phillips et al. 2005a; Richardson et al. 2003; Shikhman et al. 2004, 2001a) (Fig. 9).

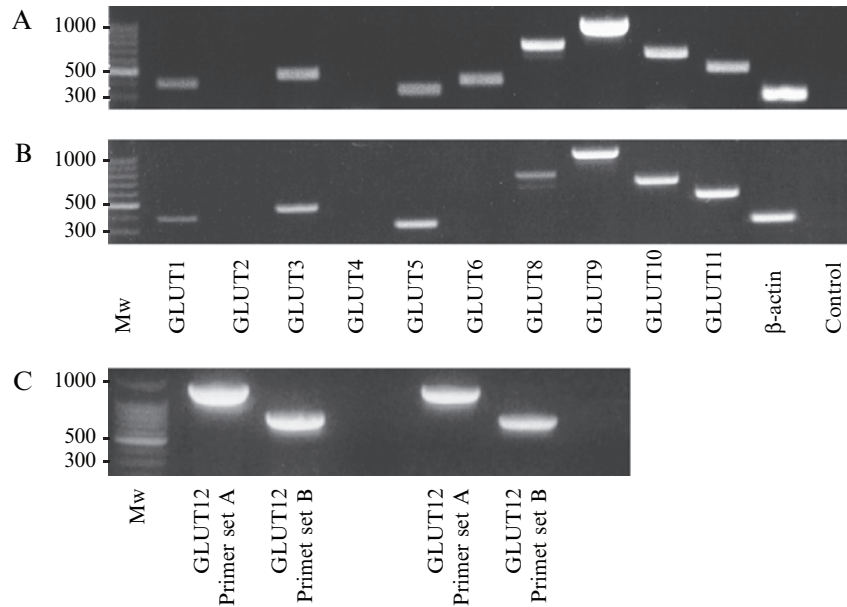


Fig. 8 Evidence for up to nine facilitative glucose transporters in human articular cartilage. **A, B** Summary of the results of RT-PCR experiments using two different human cartilage cDNA libraries which confirm the expression of GLUT1, GLUT3, GLUT5, GLUT6, GLUT8, GLUT9, GLUT10, GLUT11 and GLUT12 in cartilage. The template was omitted in the control lanes shown in **A** and **B**. **C** The presence of GLUT12 was confirmed using two different primer sets. Additional information about the sequences of the isoform specific PCR primers and the sizes of the PCR products may be found in a recent paper (Richardson et al. 2003)

7.1

Functional Significance of GLUT1 and GLUT3 in Articular Chondrocytes: The Developmental Perspective

Recent studies by our group and others suggest that GLUT1 and GLUT3 are present in chondrocytes derived from fully developed human (Fig. 10A, B), porcine (Fig. 10C), equine (Fig. 9), and ovine articular cartilage (Mobasheri et al. 2002b, 2002c; Phillips et al. 2005a; Shikhman et al. 2001a) (Figs. 11 and 12). However, the functional significance of GLUT1 and GLUT3 expression in chondrocytes has not been explored using a comparative physiology approach.

The optimal growth, development, and maintenance of musculoskeletal structures are important for skeletal stability. The availability of glucose and the expression of GLUT proteins in musculoskeletal cells are likely to influence the development of the musculoskeletal structures of load-bearing synovial joints (i.e., articular cartilage, synovium, tendon, and ligament) in all vertebrates. The facilitated transport of glu-

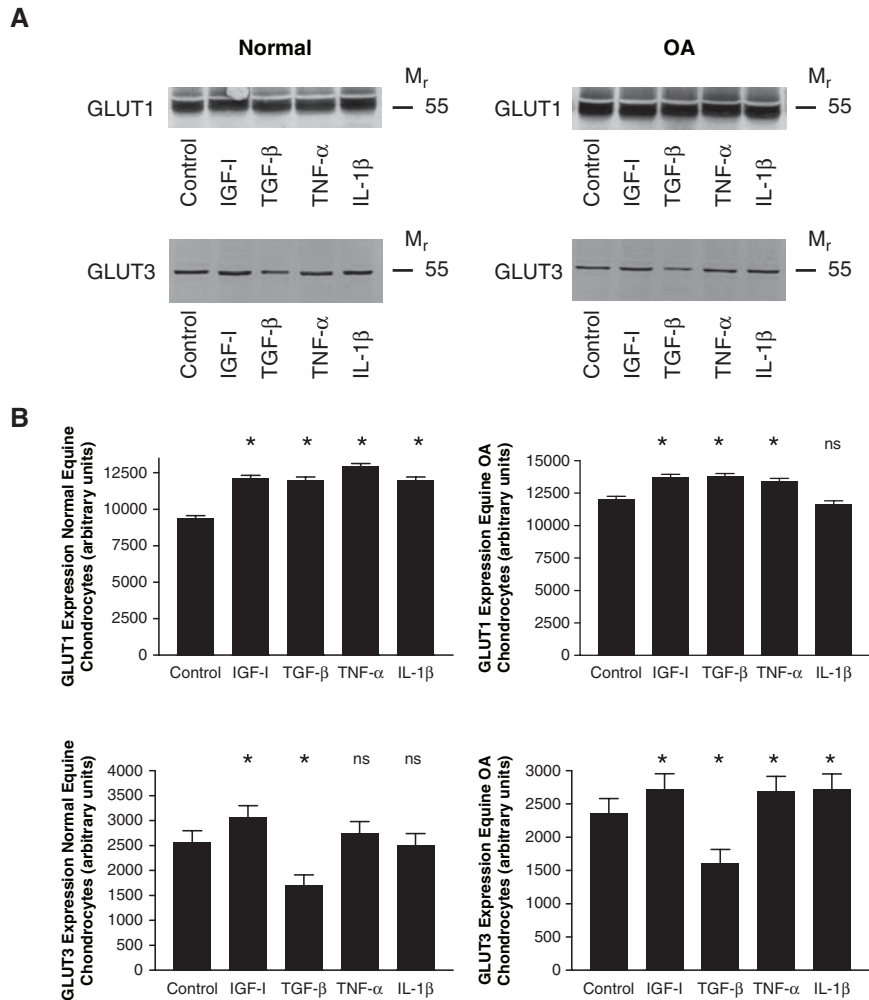


Fig.9 Evidence for growth factor and cytokine regulation of GLUT1 and GLUT3 expression in normal and osteoarthritic equine articular chondrocytes. Western blot analysis demonstrated that equine chondrocytes express GLUT1 and GLUT3. IGF-I stimulation resulted in upregulation of GLUT1 and GLUT3. TGF-β stimulation upregulated GLUT1 but downregulated GLUT3. TNF-α and IL-1β stimulation resulted in upregulation of GLUT1 but did not affect GLUT3. TGF-β also reduced the levels of the GLUT3 protein. *Denotes a significant difference ($P < 0.05$); *ns* denotes a statistically insignificant difference

cose across the chondrocyte membrane represents the rate-limiting step in glucose metabolism and is essential for the functional integrity of articulating joints (Shikhman et al. 2001a). Thorens and co-workers have reviewed and discussed the role of the mammalian GLUT/SLC2A family of glucose transporters in glucose transport (Joost

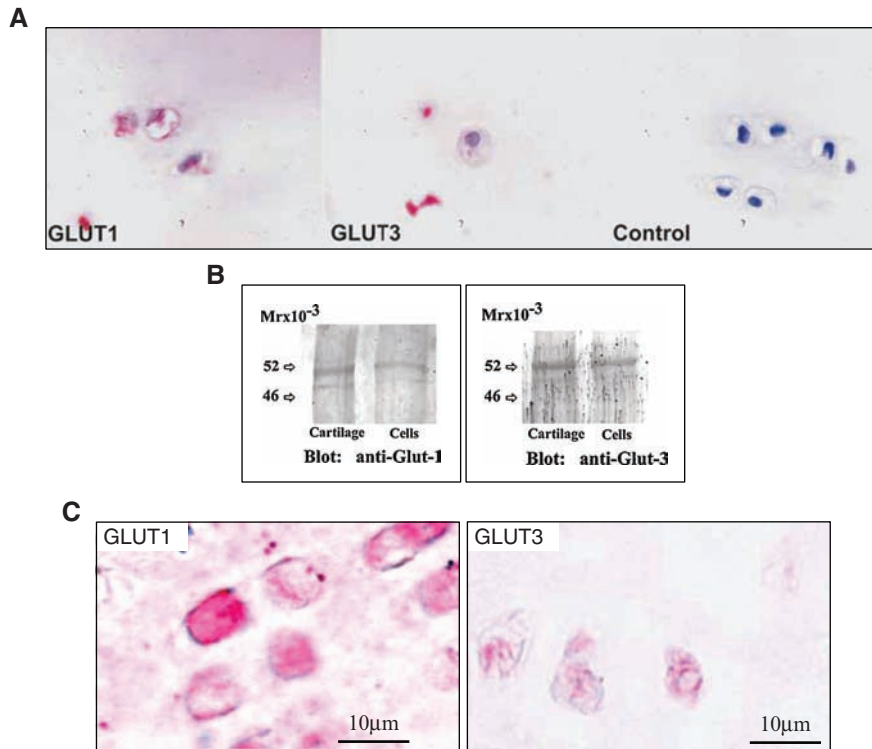


Fig. 10 GLUT1 and GLUT3 are expressed in human articular chondrocytes (A, B) and in porcine chondrocytes (C). Western blot evidence for expression of GLUT1 and GLUT3 in human cartilage and isolated chondrocytes (B). Sections of human and pig articular cartilage were incubated with polyclonal antibodies to GLUT1 and GLUT3 followed by an alkaline phosphatase-conjugated goat anti-rabbit antibody (Sigma-Aldrich) (A, C)

and Thorens 2001; Uldry and Thorens 2004). It is also important to highlight the fact that the tissue distribution of glucose transporters is not constant throughout development (Santalucia et al. 1992). High levels of GLUT1 and GLUT3 are present in a wide range of fetal tissues, but expression of these transporters greatly decreases after birth in many of these cell types. Abundant levels of the GLUT1 and GLUT3 proteins are present in pre-implantation mouse embryos, since glucose is the main substrate consumed (Pantaleon and Kaye 1998; Pantaleon et al. 2001). During the early period of organ formation (i.e., brain, heart, skeletal muscle, and kidney) GLUT1 is primarily responsible for glucose supply to the dividing and differentiating cells (Matsumoto et al. 1995; Santalucia et al. 1992). In the early organogenesis period, high-affinity glucose transporters may be required because embryonic mammalian cells may be exposed to hypoxia and may have to rely on anaerobic glycolysis (Matsumoto

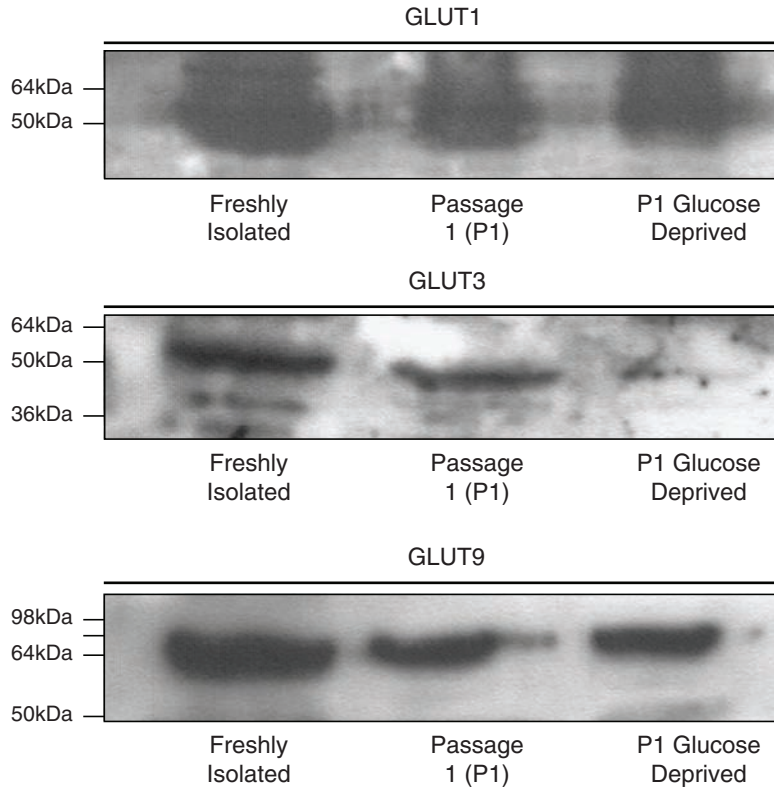


Fig. 11 Expression of GLUT1, GLUT3 and GLUT9 in whole cell lysates of freshly isolated, passage one and glucose deprived ovine articular chondrocytes. Glucose deprivation does not affect expression of GLUT1 but downregulates GLUT3 and GLUT9

et al. 1995). Glucose is particularly important for anabolic activities of the mesenchymal cells that differentiate into specialized cells of the musculoskeletal system (Vannucci and Vannucci 2000). Provision of glucose to growing musculoskeletal tissues is particularly important during fetal development, when cells are rapidly dividing and differentiating (Matsumoto et al. 1995; Pantaleon and Kaye 1998; Santalucia et al. 1992; Vannucci 1994) and involves the GLUT1 isoform (Mobasheri et al. 2005a). Many studies have shown that expression of GLUT1 and GLUT3 is greatly decreased after birth in many cell types. Studies in our laboratory have shown that GLUT1 and GLUT3 are persistently and reproducibly expressed at the mRNA and protein levels in mature chondrocytes derived from the articular cartilage of a variety of species (Mobasheri et al. 2002b, 2002c).

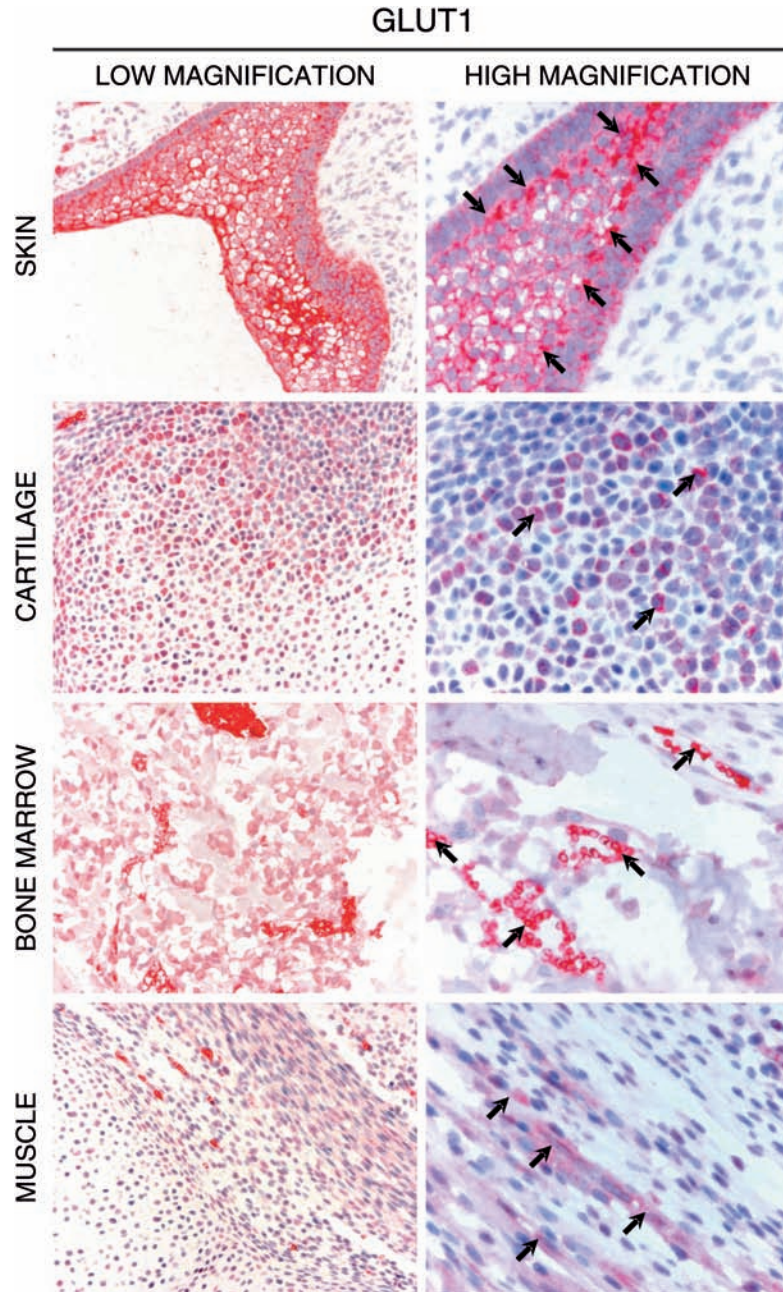


Fig. 12 Expression of GLUT1 in embryonic ovine tissues including skin, cartilage, bone marrow, and skeletal muscle. Data shown are from E42-E45 ovine embryos. See Fig. 33 for more details

7.2

Functional Significance of GLUT1 and GLUT3 in Articular Chondrocytes: The Metabolic Perspective

Studies of glucose transport and metabolism in hypoxia–ischemia in the rat brain have revealed new and valuable information about the specific roles of the GLUT1 and GLUT3 isoforms in regulating glucose uptake in low oxygen conditions. Hypoxia increases the expression of GLUT1 and GLUT3 proteins via an oxygen-sensitive transcription factor (hypoxia-inducible factor 1, HIF-1) to increase glucose transport and the glycolytic rate (Behrooz and Ismail-Beigi 1997, 1999). These proteins could also function as glucose-sensing receptors in tissues exposed to hypoxic conditions.

Cells in the central nervous system are dependent on glucose and oxygen for energy. Neurons in particular need to be buffered from fluctuations in blood glucose. Cerebral hypoxia–ischemia is known to produce major alterations in energy metabolism and glucose utilization in the brain. A number of studies have investigated the effects of hypoxia, glucose deprivation, and hypoxia plus glucose deprivation on the transcription and translation of glucose transporters in neurons (Choeiri et al. 2002) and astroglia (Morgello et al. 1995; Yu et al. 1995). The GLUT1 isoform mediates the transport of glucose across the blood–brain barrier, whereas both GLUT1 and GLUT3 mediate glucose uptake into neurons and glia (Vannucci et al. 1996). Hypoxia–ischemia in the rat brain stimulates upregulation of GLUT1 and GLUT3 glucose transporter gene expression (Vannucci et al. 1998). Animals (or cell lines) treated with the hypoxia mimetic cobalt chloride (a chemical agent that stimulates the expression of a set of hypoxia-responsive genes) also upregulate GLUT1 and GLUT3 expression (Badr et al. 1999).

Other related studies have shown that glucose deprivation alone produces minimal effects on GLUT mRNA levels in the brain, but hypoxia and glucose deprivation synergize to markedly increase GLUT gene expression (Bruckner et al. 1999). Among the various hypoxia-responsive genes, GLUT1 was the first gene whose rate of transcription was shown to be dually regulated by hypoxia (Zhang et al. 1999). It is now well appreciated that GLUT1 and GLUT3 gene expression is acutely regulated by hypoxia-inducible factor 1 (HIF-1).

Our own studies have consistently shown that the hypoxia-responsive GLUT1 and GLUT3 are functionally expressed in equine and ovine chondrocytes (Mobasheri et al. 2002b, 2002c), (Mobasheri et al. 2005a). We have recently presented a new hypothesis that implicates GLUT1 and GLUT3 glucose transporters and the hypoxia-inducible transcription factor (HIF-1 α) in glucose sensing in chondrocytes (Mobasheri et al. 2005b). The expression of GLUT1 and GLUT3 in chondrocytes may also be related to the hypoxic nature of cartilage and the unusual metabolic properties of chondrocytes (reviewed in detail in the following section).

7.3

Regulation of Hypoxia and Hypoxia-Responsive Gene Expression by the Transcription Factor HIF-1 α in Chondrocytes

Living cells are exposed to a wide spectrum of oxygenation (Fig. 13). HIF-1 is a heterodimeric basic-helix-loop-helix-PAS domain transcription factor that mediates changes in gene expression in response to changes in oxygen concentration (Wang et al. 1995a, 1995b). HIF-1 is the only known mammalian transcription factor expressed uniquely in response to physiologically relevant levels of hypoxia (Iyer et al. 1998; Semenza and Wang 1992). HIF-1 protein is normally degraded under normoxic conditions. However, despite the fact that HIF-1 mRNA levels remain the same in hypoxic conditions, HIF-1 protein is degraded less. HIF-1 activates mRNAs encoding erythropoietin and the following glycolytic enzymes: aldolase, phosphoglycerate kinase, pyruvate kinase, enolase, lactate dehydrogenase, and phosphofructokinase (Semenza et al. 1996, 1994). Other HIF-1 target genes include those encoding vascular endothelial growth factor (VEGF), and the GLUT1 and GLUT3 glucose transporters (Oquidid et al. 1999; Semenza 2001; Semenza et al. 1999; Vannucci et al. 1998, 1996) (Fig. 13 and 14). Indeed, GLUT1 and GLUT3 are early targets of HIF-1 in hypoxic conditions. The available literature in this area is extensive with much discussion of the role of HIF-1 and glucose transporters in tumor progression and we refer readers to a series of excellent articles by G.L. Semenza (Semenza 2002a, 2002b, 1998, 1999a, 1999b, 2001).

It is now well established that HIF-1 α is expressed in chondrocytes and may mediate responses to mechanical overload (Pufe et al. 2004), oxygen deprivation, and metabolic stress (Rajpurohit et al. 2002) by inducing expression of

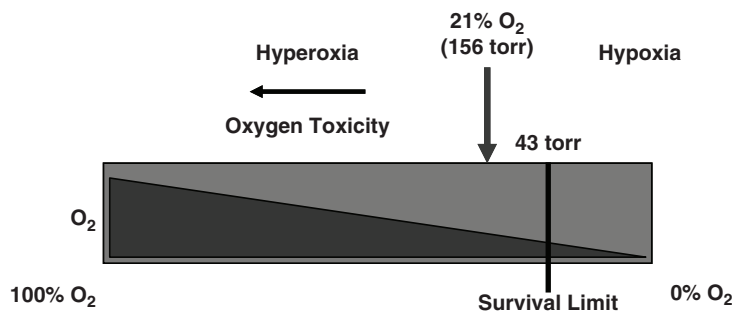


Fig. 13 Physiological and pathophysiological spectrum of oxygenation. Hyperoxia and anoxia are lethal to all cells, whereas most cells have the varied capacity to adapt to low oxygen tension hypoxia. Chondrocytes and mesenchymal stem cells appear to favor hypoxic conditions

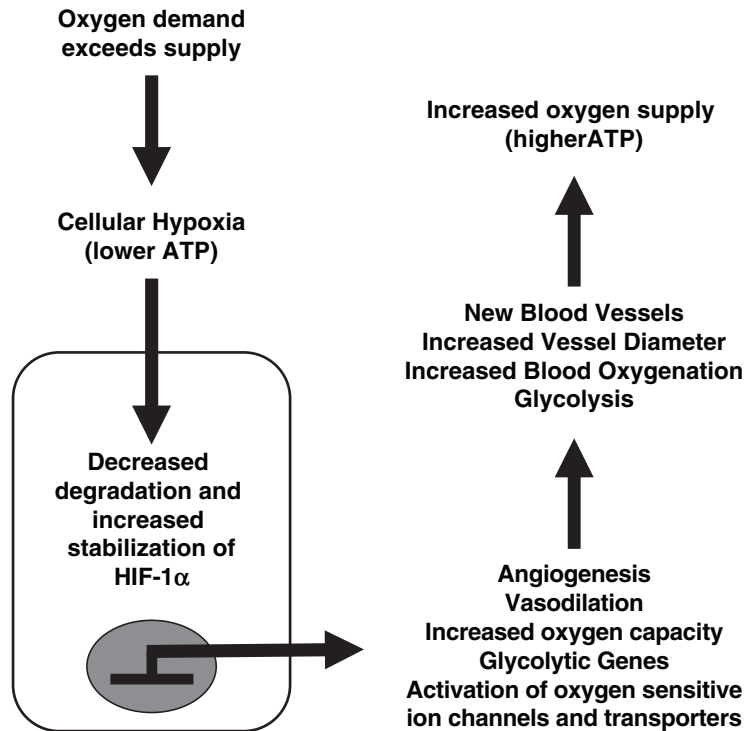


Fig. 14 Molecular responses to hypoxia; when oxygen demand exceeds supply the HIF-1 α oxygen-sensing system results in activation of key genes involved in angiogenesis, vasodilatation, oxygen delivery, and glycolysis in order to increase tissue oxygenation

VEGF and glucose transporters (Fig. 14). Studies of embryonic and epiphyseal chondrocytes have shown that HIF-1 α is essential for chondrocyte growth arrest and survival *in vivo* (Pfander et al. 2003; Schipani et al. 2001). Recent work on the nucleus pulposus (NP) in the intervertebral disc has also suggested that HIF-1 α is important for the maintenance of anaerobic glycolysis and the response to hypoxia and nutrient stress (Pfander et al. 2003; Rajpurohit et al. 2002). HIF-1 α is known to upregulate stress-responsive genes and one such gene is the vascular endothelial growth factor (VEGF). Studies of mouse epiphyseal chondrocytes have shown that soluble isoforms of VEGF, VEGF(120) and VEGF(164), are abundantly expressed splice variants in cells exposed to low oxygen levels (Cramer et al. 2004). Thus the biological effects of VEGF in low-oxygen conditions are HIF-1 α dependent since functional inactivation of HIF-1 α abolishes the hypoxic increase of VEGF expression in chondrocytes (Cramer et al. 2004). HIF-1 α may also be involved in the poorly

understood process of mechanotransduction; elegant recent studies have shown that mechanical overload increases HIF-1 α expression and immunoreactivity in cartilage which, in turn, induces VEGF expression in chondrocytes (Pufe et al. 2004). VEGF is an important growth factor for angiogenesis and vascularization but it also participates in cytokine-mediated inflammatory processes (Pufe et al. 2004, 2001). Therefore, it is becoming clear that HIF-1 α -regulated target genes are expressed in chondrocytes and are involved in diverse stress response processes.

7.4

Expression of HIF-1 α and GLUT1 in Normal and Osteoarthritic Articular Cartilage

Cells may be exposed to a variety of oxygen tensions ranging from the complete absence of oxygen, or anoxia, to super-atmospheric oxygen concentrations, or hyperoxia; both of these extremes will be toxic in the short term and lethal to living cells over longer periods of time (Fig. 13). The recent literature on cartilage metabolism suggests that chondrocytes and their precursors favor hypoxic conditions (Fig. 13 and 14). Recent immunohistochemical studies by Pfander and co-workers (Pfander et al. 2005) suggest that expression of HIF-1 protein and its target genes GLUT1 and phosphoglycerate kinase 1 (PGK-1) is increased in human chondrocytes with the severity of OA. Work from our own laboratory suggests that the hypoxia-responsive GLUT1 and GLUT3 glucose transporters and the recently described GLUT9 glucose transporter are expressed in human articular chondrocytes (Mobasheri et al. 2005b; Phillips et al. 2005a; Richardson et al., 2003) Fig. 8 and Fig. 15). Our work in human intervertebral disc cells has shown that GLUT1, GLUT3, and GLUT9 are also present in these cells (see subsequent sections). Thus, chondrocytes may depend on the adaptive functions of HIF-1 α in degenerate cartilage in order to maintain production of ATP and matrix macromolecules during the course of OA progression.

Our recent work has suggested that chondrocyte adaptation to hypoxia may occur by metabolic alterations including enhancement of the glucose transporting capacity of the cells, increased glycolysis, and lactate production. We have shown that hypoxia and glucose deprivation increase the production of lactic acid and production of the active form of MMP-2 (Mobasheri et al. 2006) (Fig. 16). Upregulation of MMP-2 and the build-up of lactate will have detrimental effects on the ECM. We have proposed that chronic hypoxia may occur in degenerate osteoarthritic joints and the consequent metabolic alterations may contribute to the pathogenesis and progression of OA.

We have observed that the glucose transport is upregulated in cultured equine chondrocytes in response to hypoxia and hypoxia mimetic agents such as cobalt chloride (Mobasheri et al. 2006) (Fig. 17). We have made very similar observations regarding the expression of GLUT1 and its regulation by hypoxia and cobalt chloride in human C-28/I2 chondrocyte-like cells (Fig. 18). GLUT1 is also regulated

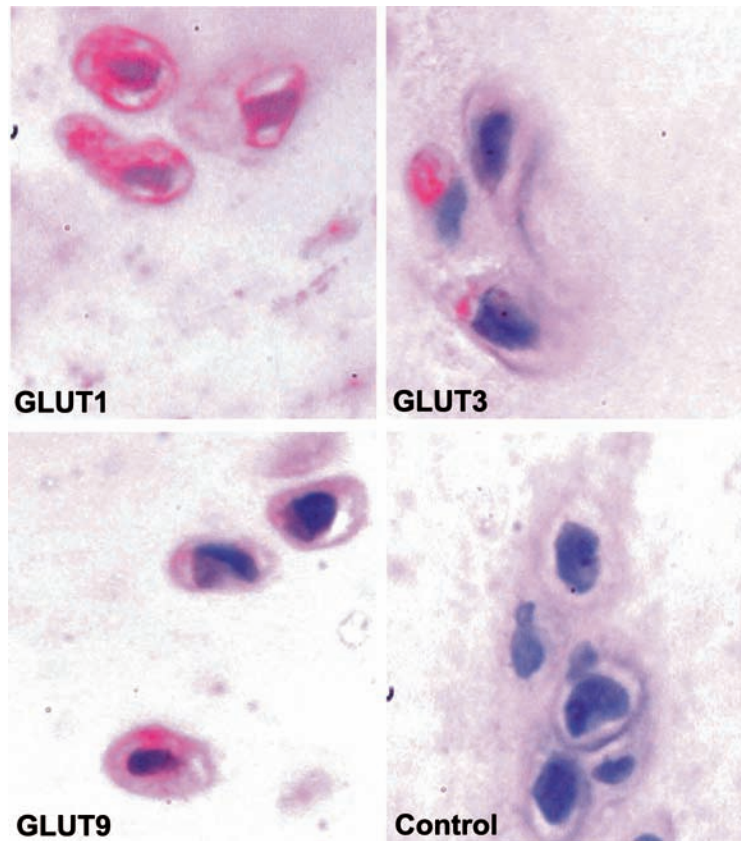


Fig. 15 Immunohistochemical evidence for expression of GLUT1, GLUT3, and GLUT9 glucose transporters in normal human articular cartilage (original magnification $\times 400$). The immunostaining for the GLUT proteins is indicated by the *red* substrate and the cell nuclei were counterstained with hematoxylin. The primary antibody was omitted in the control panel, which shows a section of human cartilage exposed only to secondary alkaline phosphatase conjugated antibody. (Reproduced from Richardson et al. 2003 with copyright permission of Elsevier Science and the OsteoArthritis Research Society International)

by glucose deprivation. As shown in Fig. 18B, GLUT1 levels are higher in C28/Ia chondrocyte-like cells deprived of glucose for 48 h than in cells cultured under normal glucose levels.

We are currently studying glucose uptake in those conditions to determine whether the lower molecular weight GLUT1 form detected is functional.

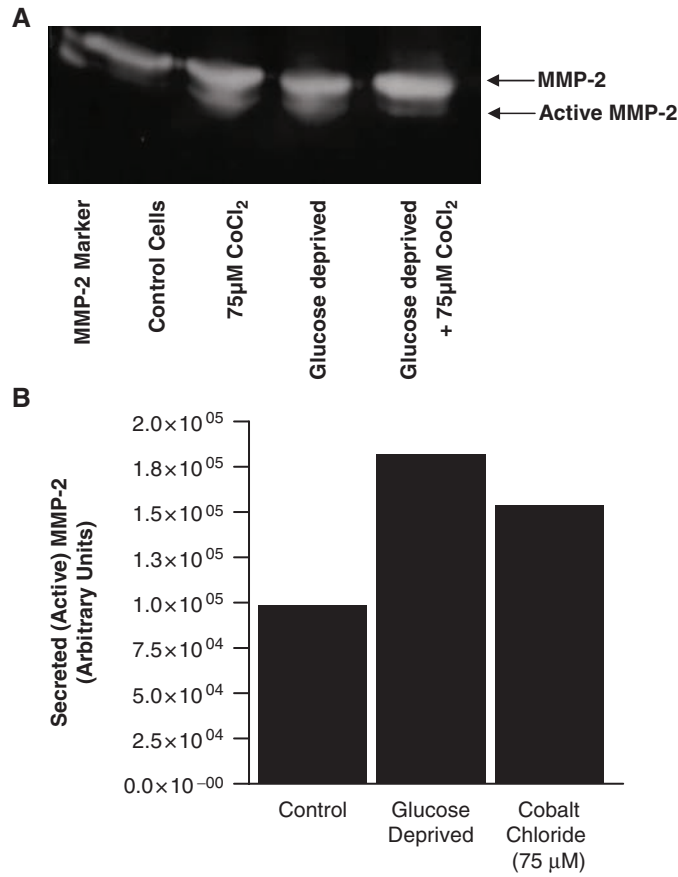


Fig. 16 Effects of glucose deprivation and cobalt chloride on the expression of active MMP-2 secreted into the culture medium of chondrocytes. **A** A representative gelatin zymogram used for the quantitative analysis of active MMP-2 expression. **B** Glucose deprivation, exposure to 75 μ M cobalt chloride or a combination of both for periods of up to 24 h significantly increased MMP-2 production secretion by chondrocytes compared to the control group. MMP-2 is detected as two closely migrating bands on the zymogram; the lower molecular weight band corresponds to active MMP-2 and the higher molecular weight band represents inactive MMP-2. (Reproduced from Mobasheri et al. 2006 with copyright permission of the New York Academy of Sciences)

These glucose transporters are also differentially regulated in response to growth factors and proinflammatory cytokines and are probably involved in energy provision for chondrocytes in inflammatory conditions (Mobasheri et al. 2005b, 2002c; Richardson et al. 2003; Shikhman et al. 2001b) (Fig. 19).

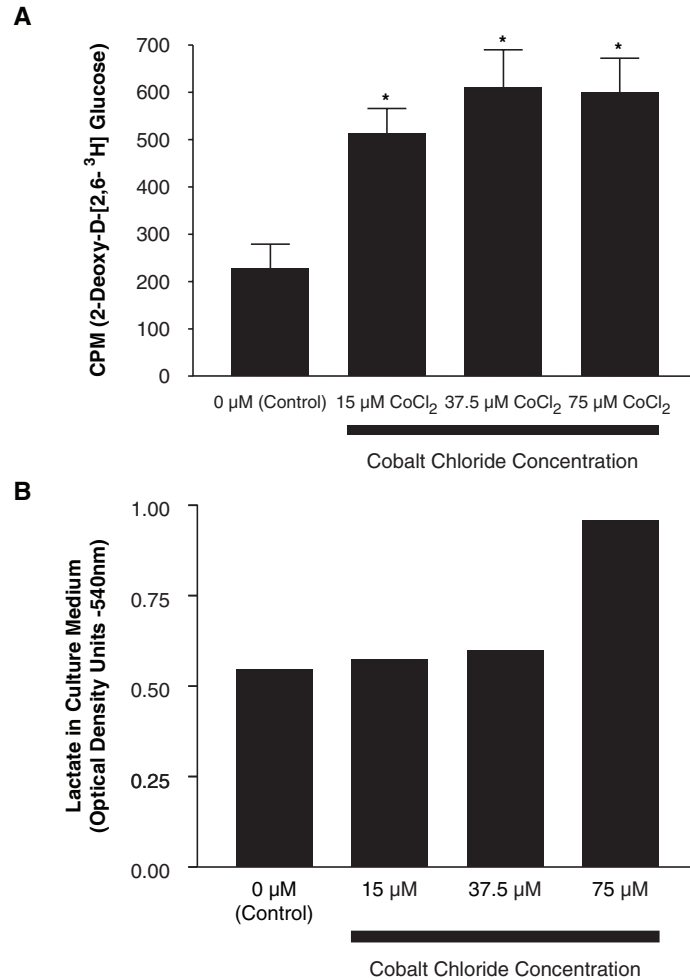


Fig. 17 A Effects of the hypoxia mimetic cobalt chloride on the uptake of 2-deoxy-D-[2, 6-³H] glucose by monolayer cultured equine chondrocytes in 24-well plates. The uptake of 2-deoxy-D-[2, 6-³H] glucose uptake in control chondrocytes was compared with cells incubated for 24 h with increasing concentrations of cobalt chloride (15, 37.5, and 75 μM). The net uptake of 2-deoxy-D-[2, 6-³H] glucose was significantly higher in chondrocytes incubated with cobalt chloride compared to control cells. The highest increase was seen with 75 μM cobalt chloride. *Error bars* indicate standard errors of the means ($n=3$). In cases where a statistically significant difference between the experimental group and a control group was found the bar is labeled with *. **B** Effects of cobalt chloride on the production of lactic acid by monolayer cultured equine chondrocytes in 24-well plates. The production of lactate in culture supernatants of control chondrocytes was compared with cells incubated for 24 h with increasing concentrations of cobalt chloride (15, 37.5, and 75 μM). Lactic acid production was higher in chondrocytes incubated with cobalt chloride compared to control cells and the effect seemed to plateau at a concentration of 75 μM cobalt chloride. (Reproduced from Mobasher et al. 2006 with copyright permission of the New York Academy of Sciences)

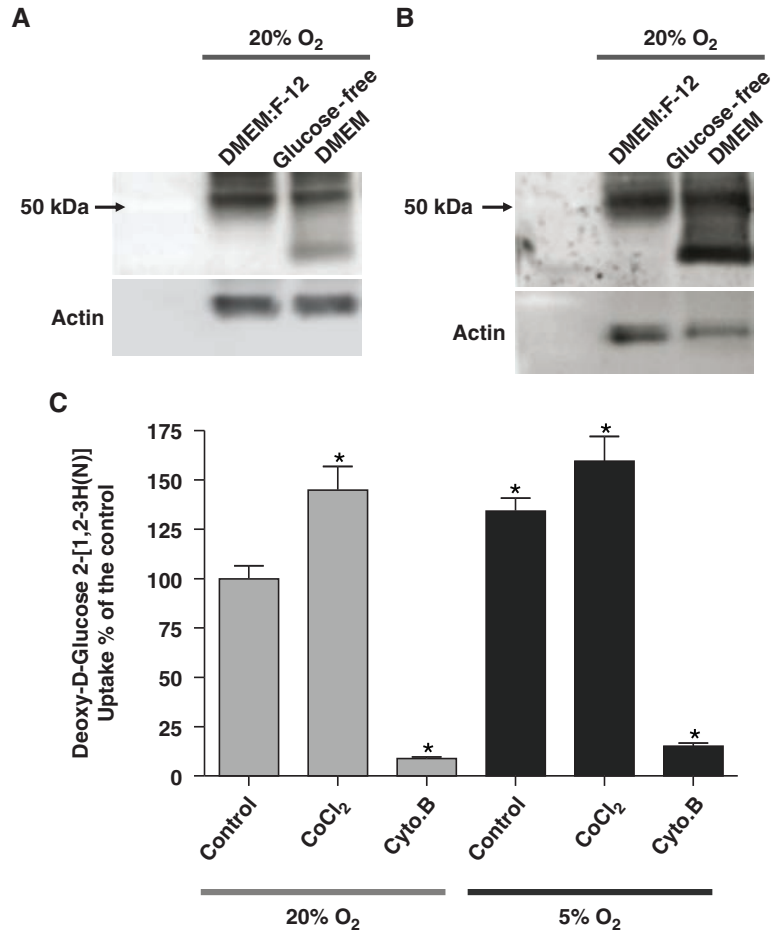


Fig. 18 Expression of GLUT1 and its regulation by glucose deprivation, hypoxia and cobalt chloride (CoCl₂) in human C-28/I₂ chondrocyte-like cells. The GLUT1 protein was detected as an approximately 50 kDa protein in whole cell extracts of C-28/I₂ chondrocyte-like cells. In cells incubated for 24 (A) or 48 h (B) in the absence of glucose, an additional band was detected corresponding to approximately 40 kDa. C Net glucose transport was significantly higher in chondrocytes maintained in hypoxia (135.33% increase compared to normoxia controls, ± 3.5 , $n = 4$); exposure of C-28/I₂ chondrocytes to CoCl₂ both in normoxia (153.24%, ± 16.7 , $n = 4$) and hypoxia (159.68% increase compared to the controls, ± 14.1 , $n = 4$) conditions also resulted in a significant increase in net glucose uptake. Statistical analysis was carried out using the one-way ANOVA test and the means were found to be significantly different. ($P = 0.0107$, significance level, $P < 0.05$)

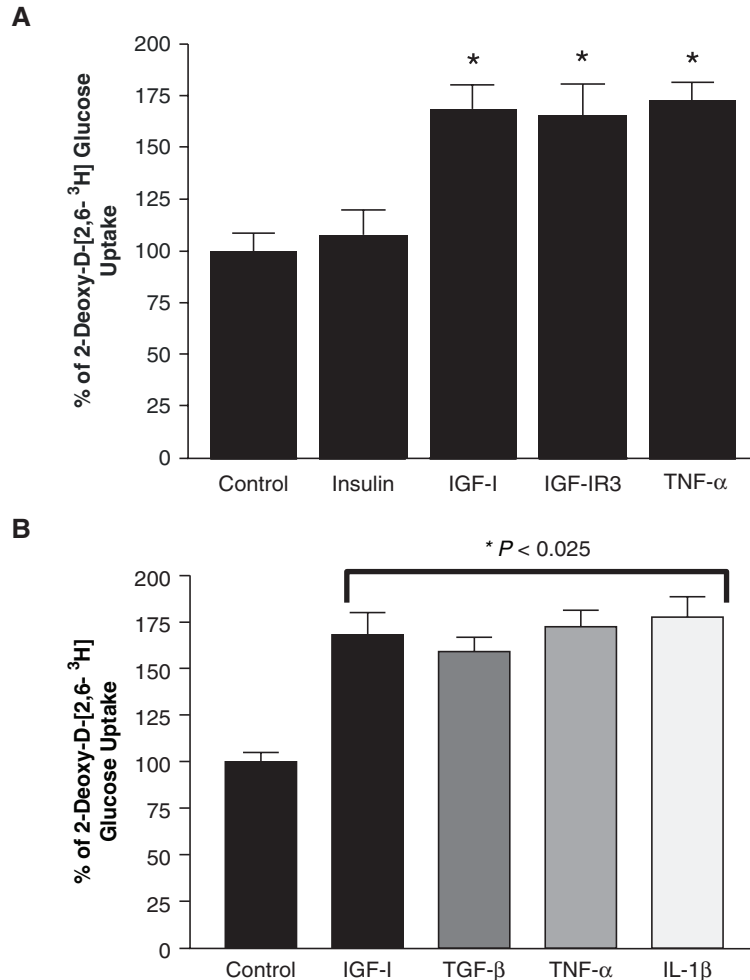


Fig. 19 A Effects of insulin, insulin-like growth factor (IGF-I) and TNF- α on 2-deoxy-D-[2, 6-³H] glucose uptake by C20/A4 chondrocytes. Chondrocytes were stimulated with insulin (12.5 $\mu\text{g ml}^{-1}$), TNF- α (20 ng ml^{-1}), and recombinant (long R3) IGF-I (20 ng ml^{-1}) for a period 24 h at 37°C before facilitated glucose transport was measured by 2-deoxy-D-[2, 6-³H] uptake. Baseline 2-deoxy-D-[2, 6-³H] uptake in un-stimulated chondrocytes (control) was considered as 100%. Error bars indicate standard errors of the means ($n=3$). In cases where a statistically significant difference between the experimental group and a control group was found the bar is labeled with *. (Reproduced from Richardson et al. 2003 with copyright permission of Elsevier Science and the OsteoArthritis Research Society International). **B** Uptake of 2-deoxy-[2, 6-³H] glucose by equine articular chondrocytes stimulated with IGF-I, TGF- β , TNF- α and IL-1 β . Baseline 2-deoxyglucose uptake in un-stimulated chondrocytes (control cells) was considered as 100%. Error bars indicate standard errors of the means ($n=3$). An asterisk denotes a significant difference between control and experimental groups ($P < 0.025$). (Reproduced from Phillips et al. 2005b with copyright permission of Elsevier Science)

7.5

Functional Significance of ATP-Sensitive (K_{ATP}) Potassium Channels in Articular Chondrocytes

As we have discussed in the previous section, articular cartilage is an avascular and hypoxic connective tissue in which the availability of oxygen and glucose is limited and depends primarily on diffusion from the synovial microcirculation and, to a lesser extent, subchondral blood vessels (Coimbra et al. 2004; Mobasheri et al. 2005b; Schipani et al. 2001). In a previous section we discussed the importance of subchondral blood vessels and their physical separation from the articular cartilage making it difficult for them to participate in the provision of nutrients to articular cartilage nutrition. Chondrocytes are glycolytic cells and are able to survive in an ECM with limited nutrients and low oxygen tensions (Henrotin et al. 2005a; Mobasheri et al. 2005b). Consequently, chondrocytes must have the capacity to sense the available levels of nutrients and ATP in the intracellular and extracellular compartments and respond appropriately by adjusting cellular metabolism and ATP production levels (Edwards and Weston 1995).

Potassium channels are integral membrane proteins present in most mammalian cells. They participate in a wide range of physiological responses including control of the cell membrane potential in excitable cells in the brain and the pancreas and regulating contractile tone in a variety of muscle types (cardiac, skeletal, and vascular smooth muscle) (Edwards and Weston 1995). Opening of potassium channels hyperpolarizes membranes and promotes quiescence, whereas their closure produces depolarization and excitation (Christie 1995; Coetzee et al. 1999). A large superfamily of potassium channels has been identified, including: voltage-activated potassium channels (K_v), Ca^{2+} -activated potassium channels (K_{Ca}), and inward rectifier potassium channels (K_{ir}) (Babenko et al. 1998). K_{ATP} channels are members of the K_{ir} family, existing as a complex of an ATP-binding protein (SUR) and a channel component ($K_{ir}6.x$) (Minami et al. 2004; Quayle et al. 1997). They are widely distributed in neuroendocrine, pancreatic, cardiac, skeletal, and smooth muscle cells; it is believed that they serve to couple metabolic state to cellular activity. This coupling is important in both physiological and pathological conditions (Dart and Standen 1993, 1995).

Recent studies on ion channels in chondrocytes have expanded our understanding of the roles of these proteins in chondrocyte and cartilage function. Nevertheless, data on chondrocyte ion channels are still very limited compared to other well-studied cell types. Despite the physiological importance of potassium channels in the modulation of metabolic activity, there are significantly fewer publications relating to potassium channel expression and function in chondrocytes compared to other tissues. Low oxygen tension and hypoxia are known to lead to activation of K_{ATP} channels in other systems (Phillis 2004; Dart and Standen 1993, 1995) suggesting that these channels are important in hypoxia-mediated cell signaling (Mobasheri et al. 2007). In a recent study we used a combined electrophysiological and immunohistochemical approach to test the hypothesis that K_{ATP} channels are

expressed in articular chondrocytes and may therefore be involved in metabolic regulation. We used the patch-clamp technique to investigate whether K_{ATP} channels are functionally expressed in isolated equine articular chondrocytes and employed immunohistochemistry to determine the expression of $Kir_{6.1}$ in human and equine chondrocytes from both normal and OA cartilage (Mobasheri et al. 2007). The results of this study show, for the first time, that K_{ATP} channels ($Kir_{6.1}$ subunit of the channel) are present in normal and OA chondrocytes from equine and human subjects (Fig. 20). Furthermore, we have demonstrated that K_{ATP} channels are functionally expressed in equine chondrocytes (Fig. 21). In view of their function in other cell types (Fig. 22), we have proposed that these potassium ion channels may be important in the regulation of cartilage metabolism and intracellular ATP sensing (Fig. 23) (Mobasheri et al. 2007; Pfander et al. 2003; Rajpurohit et al. 2002; Schipani et al. 2001). In the pancreatic β cell the K_{ATP} channel senses metabolic changes in the cell, thereby coupling metabolism to electrical activity and ultimately to insulin secretion (Ashcroft and Gribble 1998). When K_{ATP} channels open, β cells hyperpolarize and insulin secretion is suppressed. We propose that in a situation

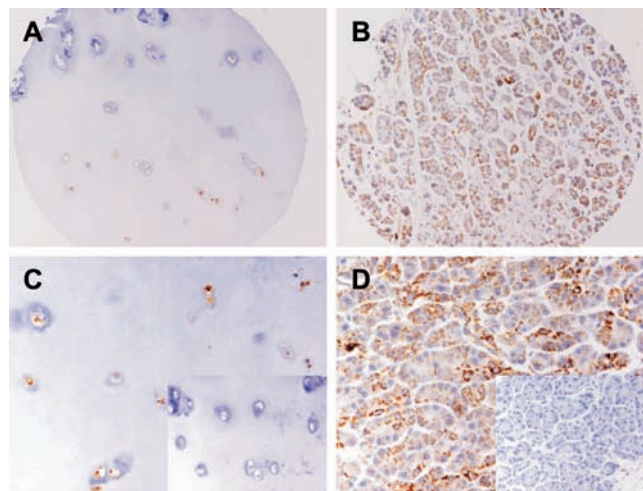


Fig.20 $Kir_{6.1}$ is expressed in chondrocytes in normal human articular cartilage. Immunohistochemical analysis of $Kir_{6.1}$ expression in samples of full-depth human articular cartilage and human pancreas represented on the CHTN2002N1 multiple human Tissue MicroArrays (TMAs). Incubation of CHTN2002N1 TMAs with polyclonal antibodies to $Kir_{6.1}$ followed by horseradish peroxidase-labeled rabbit anti-goat IgG (DakoCytomation) produced positive immunostaining in chondrocytes in human knee cartilage (low magnification shown in A, high magnification shown in C) and human pancreas (low magnification shown in B, high magnification shown in D). Omission of primary antibody from the immunohistochemical procedure resulted in complete abrogation of specific immunostaining of human articular chondrocytes in cartilage samples (*inset*, C) and pancreatic cells (*inset*, D). (Reproduced from Mobasheri et al. 2007 with copyright permission of Elsevier Science and the OsteoArthritis Research Society International)

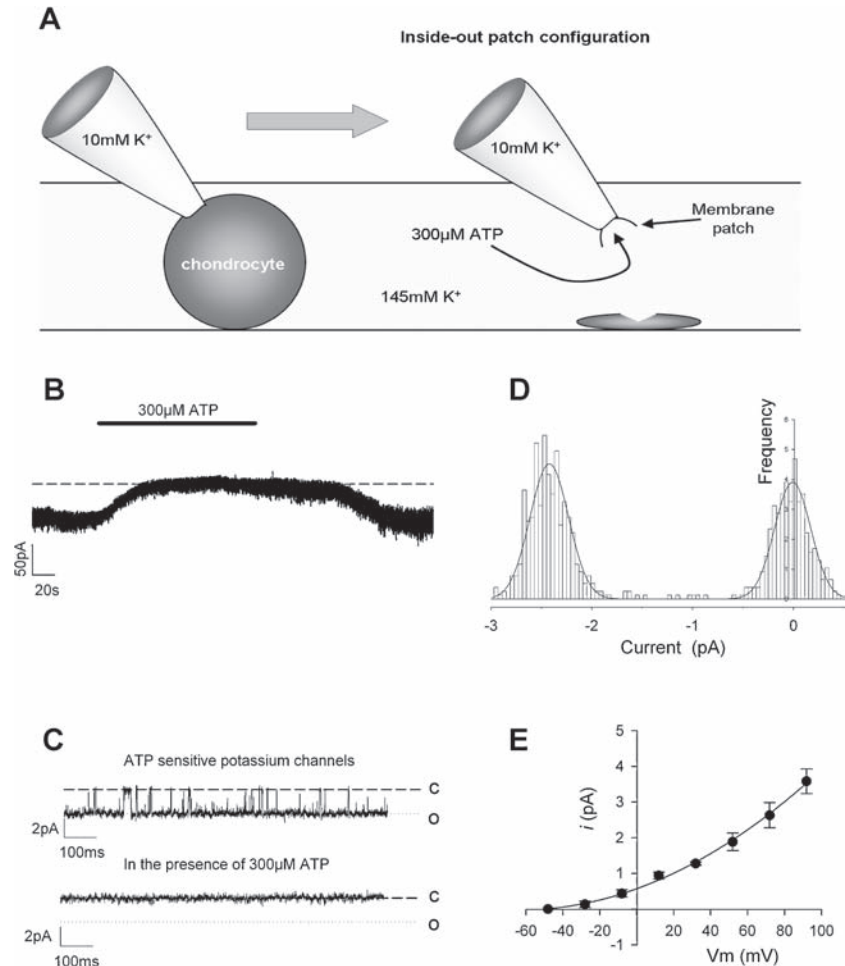


Fig. 21 Chondrocytes express functional ATP-sensitive potassium channels. **A** Outline of experimental design, for further details see (Mobasheri et al. 2007). **B** Addition of 300 μ M ATP (added by bath perfusion) inhibits currents in inside-out maxi-patches of equine articular chondrocytes. *Dashed line* indicates the zero current level, holding potential -60 mV. **C** Inside-out single-channel patch in the absence (*upper panel*) and presence (*lower panel*) of 300 μ M ATP. The *dashed line* represents the zero current level, where the channel is closed ("C") and the *dotted line* represents the unitary current level, where the channel is open ("O"). Holding potential -60 mV. **D** All points amplitude histogram from the patch shown in **C**. **E** Current-voltage curve for ATP-sensitive potassium channels recorded in a number of experiments similar to **C** and **D**. (Reproduced from Mobasheri et al. 2007 with copyright permission of Elsevier Science and the OsteoArthritis Research Society International)

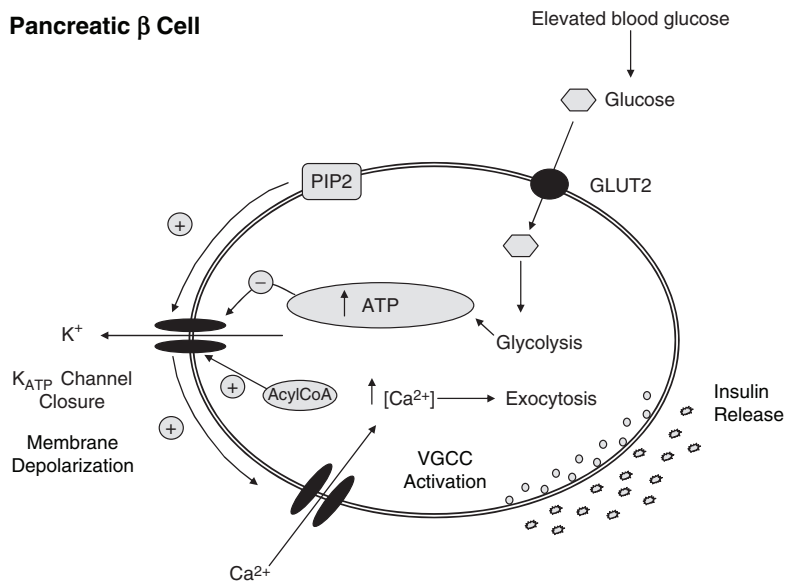


Fig. 22 The role of the pancreatic β cell K_{ATP} channel in secretion of insulin in response to elevated blood glucose levels. A rise in blood glucose is an important metabolic signal that closes K_{ATP} channels, causing membrane depolarization, activation of voltage gated calcium channels (VGCC), free calcium entry and insulin release by exocytosis. It is thought that various additional effectors including phosphatidylinositol-4,5-bisphosphate (PIP₂) and acyl CoAs modulate the ATP sensitivity of the K_{ATP} channel thereby affecting the coupling of pancreatic cell metabolism to insulin secretion. (Adapted from Koster et al. 2005)

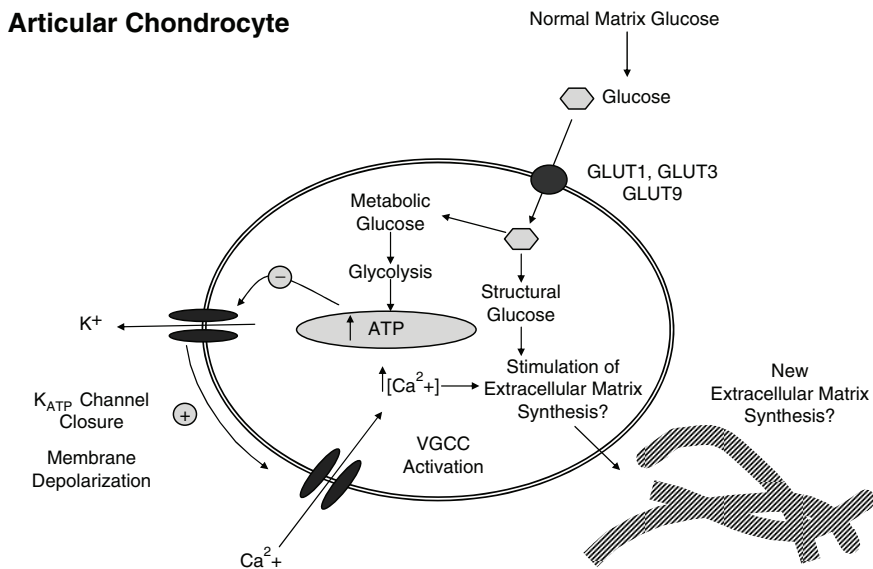


Fig. 23 Proposed role of the chondrocyte K_{ATP} channel in metabolic regulation and intracellular ATP sensing. In this hypothetical model the K_{ATP} channel may be part of extracellular glucose and intracellular ATP sensing machinery of the chondrocyte. Adequate provision of glucose ensures optimal glucose levels for the distinct metabolic and structural pools of glucose within chondrocytes which will promote anabolic processes including extracellular matrix synthesis

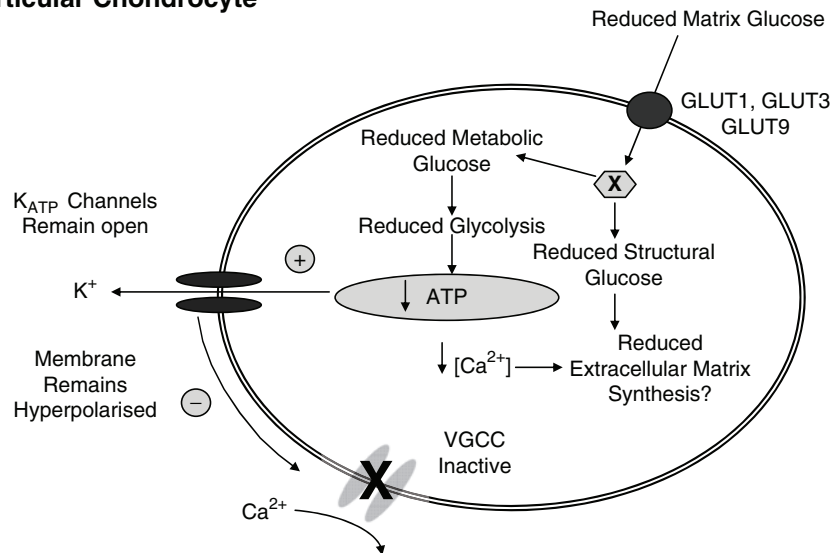
Articular Chondrocyte

Fig. 24 Putative contribution of the chondrocyte K_{ATP} channel to sensing reduced matrix and intracellular ATP levels. In this scenario a deficiency or shortfall in glucose delivery to chondrocytes may impede anabolic processes and result in a net reduction in extracellular matrix synthesis

where the supply of glucose to the synovial joint is affected (i.e., in metabolic disease), this may affect the process of metabolic regulation which may reduce ECM synthesis or affect matrix turnover (Fig. 24). Further experiments are underway to investigate the mechanisms of extracellular glucose and intracellular ATP sensing by chondrocytes and whether these are perturbed in disease, thus impairing optimal metabolic regulation and extracellular glucose sensing.

8 Regulation of Glucose Transport by Nonsteroidal Anti-inflammatory Drugs

Cartilage destruction in arthritis and OA is linked to aberrant proinflammatory cytokine and growth factor expression in the joint (Chikanza and Fernandes 2000; Malemud et al. 2003). The proinflammatory cytokines $TNF-\alpha$ and $IL-\beta$ have been found in significantly elevated levels in the synovial fluid of OA joints (Goldring 1999, 2000a; van den Berg 1999). Catabolic pathways are activated by $TNF-\alpha$ and $IL-\beta$, which are both upregulated in OA (Malemud et al. 2003). These proinflammatory mediators cause an increase in cartilage matrix degradation through increased MMP and aggrecanase activity in the joint. In addition, $TNF-\alpha$ and

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