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Review

Glucose transport and metabolism in chondrocytes: a key to understanding chondrogenesis, skeletal development and cartilage degradation in osteoarthritis

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Summary. Despite the recognition that degenerative cartilage disorders like osteoarthritis (OA) and osteochondritis dissecans (OCD) may have nutritional abnormalities at the root of their pathogenesis, balanced dietary supplementation programs have played a secondary role in their management. This review emphasizes the importance and role of nutritional factors such as glucose and glucose-derived sugars (i.e. glucosamine sulfate and vitamin C) in the development, maintenance, repair, and remodeling of cartilage. Chondrocytes, the cells of cartilage, consume glucose as a primary substrate for ATP production in glycolysis and utilize glucosamine sulfate and other sulfated sugars as structural components for extracellular matrix synthesis and are dependant on hexose uptake and delivery to metabolic and biosynthetic pools. Data from several laboratories suggests that chondrocytes express multiple isoforms of the GLUT/SLC2A family of glucose/polyol transporters. These facilitative glucose transporter proteins are expressed in a tissue and cell-specific manner, exhibit distinct kinetic properties, and are developmentally regulated. They may also be regulated by endocrine factors like insulin and insulin-like growth factor I (IGF-I) and cytokines such as interleukin 1 beta (IL-1ß) and tumour necrosis factor alpha (TNF-α). Recent studies suggest that degeneration of cartilage may be triggered by metabolic disorders of glucose balance and that OA occurs coincident with metabolic disease, endocrine dysfunction and diabetes mellitus. Based on these metabolic, endocrine and developmental considerations we present a novel hypothesis regarding the role of glucose transport and metabolism in cartilage physiology and pathophysiology and speculate that supplementation with sugar-derived vitamins and nutraceuticals may benefit patients with degenerative joint disorders.

Key words: Cartilage, Chondrocyte, Chondrogenesis, Osteoarthritis, Osteochondritis dissecans, Glucose, GLUT/SLC2A family, glucosamine sulfate, IGF-1, Vitamin C, Nutraceutrical, Glucose transporter

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Introduction

Two years before the end of the last millennium, we published an extensive review of ion transport in articular chondrocytes in an effort to stimulate, encourage and consolidate research efforts in this important area of connective tissue biology (Mobasheri et al., 1998). The representation in Fig. 1 summarizes our current understanding of the ion transport systems involved in the maintenance of the ionic milieu of chondrocytes; a variety of ion transport mechanisms are present in chondrocytes that are well placed to maintain the intracellular homeostasis of chondrocytes in face of the unusually harsh extracellular matrix (ECM) of cartilage. Membrane transport systems play critical roles in cell physiology and intracellular homeostasis in articular chondrocytes and these systems must therefore be important in maintaining cartilage integrity. Indeed, most of the plasma membrane transport systems identified in chondrocytes already are pharmacological targets for drugs used to treat various disorders of the central nervous system (Catterall, 1999; Yamakura et al.,

2001), the heart (Grant and Wendt, 1992; Dumaine and Kirsch, 1998), the stomach (Sachs and Wallmark, 1989; Sachs, 2001), the gastrointestinal tract (Kirsten et al., 1998), the kidneys (Lant, 1986, 1987; Beitz and Schulz, 1999) and the liver (Kirsten et al., 1998).

A decade ago, very little was known about nutrient transport in chondrocytes, particularly the transport of glucose and related sugars, which are essential for the synthesis of glycosaminoglycans by chondrocytes. The only published information at that time related the importance of glucose as a metabolic substrate (Otte, 1991) and emphasized the fact that glucose uptake is stimulated by catabolic cytokines in fibroblasts (Bird et al., 1990) and chondrocytes (Hernvann et al., 1992) and that stimulated glucose uptake is inhibited by anti-inflammatory cortisol (Hernvann et al., 1992, 1996). The last three years have seen significant progress in this area although perhaps not compared to advances in our knowledge in other "soft" tissues. Therefore, the time has arrived in chondrocyte cell biology for recapitulation. Novel information gained about the physiological roles of transporters, particularly those involved in glucose uptake for essential metabolic and biosynthetic reactions in cartilage, and their regulation by tissue factors (growth factors and cytokines) and hydrostatic pressure, may contribute to a better understanding of the altered molecular and cellular mechanisms in cartilage pathologies such as osteochondritis dissecans (OA) and osteoarthritis (OCD). Accordingly, the aim of this paper is to review the recently published information on glucose metabolism and transport in chondrocytes and to critically evaluate recent developments in the novel area of cartilage nutraceutical research in an effort to determine if plasma membrane transport systems responsible for glucose uptake in chondrocytes and endocrine-regulated metabolic mechanisms and signalling pathways may offer suitable targets for modulating the behaviour and biosynthetic activity of articular chondrocytes. Future progress in dealing with degenerative joint disorders such as OA and OCD will be highly dependent on a better understanding of the unique nutritional requirements of chondrocytes. A clearer knowledge of chondrocyte nutrition and the regulation of transport systems responsible for nutrient uptake in chondrocytes in health and disease may reveal underlying metabolic disturbances that are directly responsible for cartilage degradation in OA and other arthropathies. This knowledge will lead to new approaches and novel therapies to prevent and treat degenerative joint disease and enhance the approach for the discovery and design of drugs capable of modifying degenerative joint diseases.

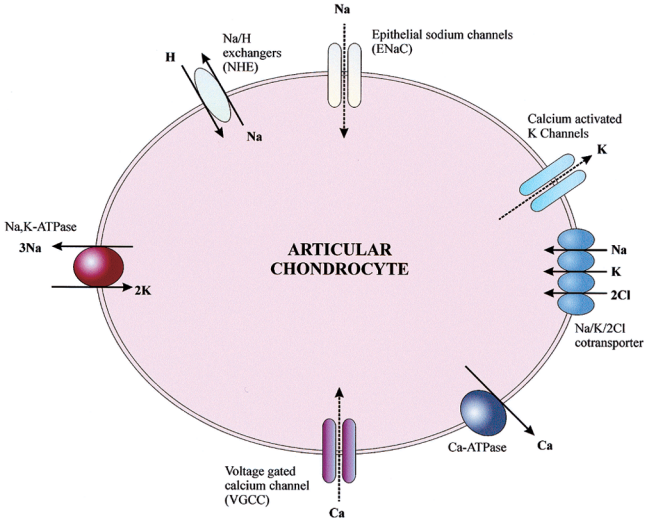


Fig. 1. Ion transport systems identified in articular chondrocytes. The principal known ion channels and transport systems (pumps and exchangers) in chondrocytes are illustrated. Na^+, K^+-ATPase: Mobasheri et al., 1997a/b; Trujillo et al., 1999a. Ca^{2+}-ATPase; Mobasheri et al., 1998. Na^+/H^+ exchange (NHE): Trujillo et al., 1999b. $Na^+/K^+/2Cl^-$ co-transporter: Trujillo et al., 1999b. Epithelial Na^+ channels (ENaC): Mobasheri and Martín-Vasallo, 1999; Trujillo et al., 1999b. Calcium activated K^+ channels: Grandolfo et al., 1992; Voltage gated calcium channels: Zuscik et al., 1997; Wang et al., 2000; Shakibaei and Mobasheri, 2000; Mobasheri et al., 2002b.

Structure and function of articular cartilage

Articular cartilage is an avascular, aneural and alymphatic connective tissue designed to distribute mechanical load, and provide a wear resistant surface to articulating joints (Buckwalter and Mankin, 1998a-c). Chondrocytes are the only cells found within articular cartilage and they are responsible for synthesizing and degrading an ECM of type II collagen, non-collagenous proteins and proteoglycans. Despite their relatively homogeneous phenotype, chondrocytes vary in size, morphology and metabolic activity depending on which zone of articular cartilage they are found (Figs. 2, 3). These specialized cells surround themselves with a territorial, pericellular ECM, primarily responsible for mechanotransduction (Guilak, 2000; Mobasheri et al., 2002b) and an inter-territorial ECM that makes up the bulk of the load-bearing tissue (Fig. 3G). When isolated and maintained in monolayer culture (Fig. 3E,F), these cells rapidly (after 4-5 passages) lose their fully differentiated functions and de-differentiate into a fibroblastic phenotype. However, when they are grown in 3-dimensional culture de-differentiated chondrocytes re-express the differentiated phenotype (Benya and Shaffer, 1982).

Changes in cartilage structure and function in osteoarthritis (OA)

Loss of cartilage is the major cause of joint dysfunction and disability in human and animal OA (Buckwalter and Mankin, 1998c; Buckwalter et al., 2000). Primary OA is the most common non-inflammatory arthropathy of both humans and animals

as a disorder of movable joints, particularly those that are large and weight bearing. OA is grossly characterized by aberrant synthesis of articular cartilage matrix, gradual hypocellularity, eventual fragmentation and degradation of articular cartilage, peri-articular new bone formation (osteophytosis), decreased, then increased, subchondral bone density and variable synovial inflammation (Buckwalter and Mankin, 1998c; Buckwalter et al., 2000). Furthermore, OA is the clinical phenotype resulting from a number of possible abnormalities of connective tissue function combined with aberrant chondrocyte behaviour and an overwhelming of the cartilage's reparative abilities. The pathological changes observed in OA appear to follow cellular and molecular processes involving catabolic and reparative events. In OA, mechanical stress initiates cartilage lesions by altering chondrocyte-matrix interaction and metabolic responses in the chondrocytes (Goldring, 2000a). There are initial increases in the amounts of water and proteoglycans associated with the observed transient chondrocyte proliferation of early OA. Proliferating chondrocytes appear in clusters or islands and are accompanied by a change in cellular organisation, indicating their undifferentiated nature. In contrast collagen type X, which is normally produced by terminally differentiated hypertrophic chondrocytes, has been demonstrated surrounding chondrocyte clusters in osteoarthritic cartilage (von der Mark et al., 1992). Chondrocyte proliferation is considered to be an attempt to counteract cartilage degradation but disease progression and secondary inflammation proves that this is generally unsuccessful. The short-lived hyperplasia (chondrocyte cloning) is followed by hypocellularity and apoptosis (Blanco et al., 1995, 1998; Mobasheri, 2002). Catabolic events responsible for cartilage matrix degradation comprise the release of catabolic cytokines such as IL-1ß, IL-6 and TNF-α inducing matrix degrading enzymes such as matrix metalloproteinases (MMPs) and aggrecanase (ADAM-TS4, ADAM-TS11) by chondrocytes and by synoviocytes in early OA (Martel-Pelletier, 1998; Goldring, 1999, 2000b). Imbalance between MMPs and tissue inhibitors of MMPs occurs, resulting in active MMPs (Fig. 4) and this may be important in cartilage matrix degradation. However, IL-1ß may also contribute to the depletion of cartilage matrix by decreasing synthesis of cartilage specific proteoglycans and collagen type II (Taskiran et al., 1994; Studer et al., 1999; Goldring, 2000b; Richardson and Dodge, 2000; Robbins et al., 2000). Systemic effects of elevated IL-1ß levels include stimulation of glucose transport and metabolism causing hypoglycaemia and impairing glucose-induced insulin secretion (Del Rey and Besedovsky, 1987). In articular cartilage, the acute effects of IL-1ß also involve stimulated glucose uptake and metabolism (Hernvann et al., 1996; Shikhman et al., 2001b). When the matrix is degraded, an inappropriate, inferior repair matrix is synthesized which cannot withstand mechanical load. Consequently, cartilage fibrillation and breakdown occurs by the focal formation of vertical, oblique and tangential clefts into the ECM and is localized preferentially in areas of proteoglycan depletion. Apoptosis is another contributing factor to the loss of articular cartilage in OA: apoptosis increases the cell loss observed in aging and OA cartilage (Adams and Horton, 1998; Blanco et al., 1998; Mobasheri, 2002). In addition to deregulated MMP activity and chondrocyte apoptosis, poor diets and malnutrition are also considered to be contributors to the pathogenesis of bone and joint disorders in human and animals (Kealy et al.,

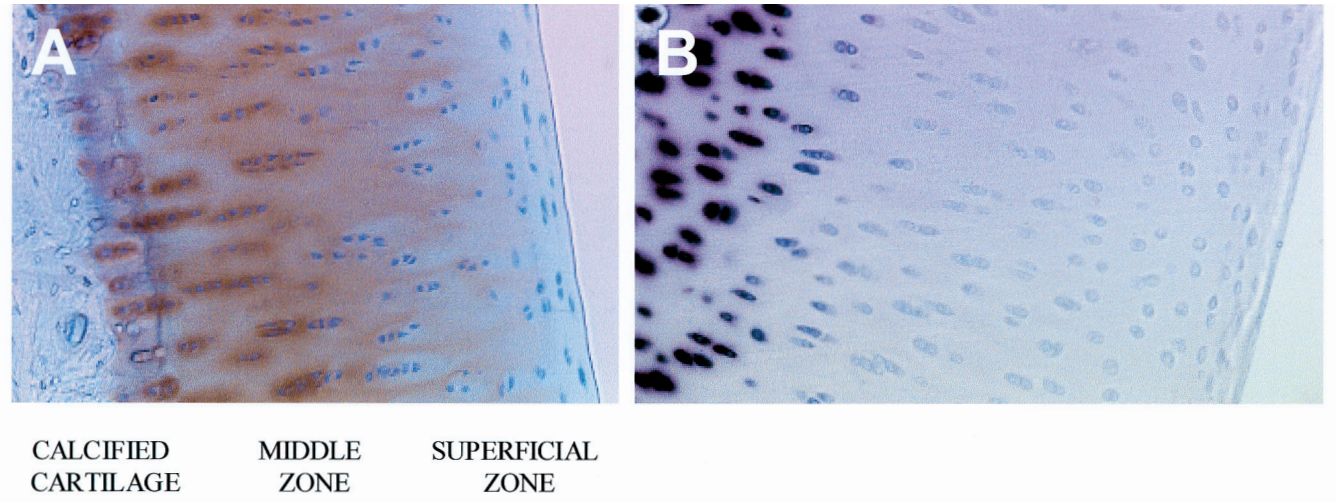


Fig. 2. A. Micrograph of Nissl stained bovine articular cartilage from the metacarpal phalangeal joint showing the organization of the tissue and zonal distribution of chondrocytes. **B.** Expression of alkaline phosphatase; a marker of matrix mineralization and calcification in chondrocytes from the deep zone of developing cartilage. x 200

1997; McAlindon and Felson, 1997).

Regulation of cartilage turnover by nutritional and endocrine factors

Levels of circulating IFG-I and IGF-I binding proteins (IGFBPs) are decreased in cases of chronic undernutrition (Underwood, 1996). Dietary manipulation can have a significant effect on IGFBPs such as reduction in IGFBP-3, the principal carrier of circulating IGF-I in serum and under adverse nutritional conditions IGFBP-1 and IGFBP-2 are also affected (Underwood, 1996). These changes in IGF-I distribution levels and its clearance will have significant effects on cartilage turnover. Recent studies also suggest that human articular chondrocytes possess the leptin receptor (a product of the obese *Ob-R* gene), and that this receptor is present on chondrocytes in articular human cartilage *in situ* (Figenschau et al., 2001). Binding of leptin to Ob-R results in phosphorylation of signal transducers and activators of transcription factors (STAT1 and STAT5) and chondrocytes stimulated with leptin exhibit increased proliferation and enhanced synthesis of ECM macromolecules (proteoglycans and collagen). Leptin may also play an important role in endochondral ossification; high levels of leptin expression have been detected in hypertrophic chondrocytes adjacent to capillary blood vessels invading hypertrophic cartilage (Kume et al., 2002). These results suggest that leptin affects cartilage generation directly and that circulating leptin exerts its influence on endochondral ossification by regulating angiogenesis. This is a novel role for leptin in skeletal growth and development. Collectively, these studies identify a role for nutrition, circulating IGFs, IGFBPs and leptin in cartilage development and physiological remodelling of cartilage matrix.

Endocrine dysfunction and associated osteoarticular disorders

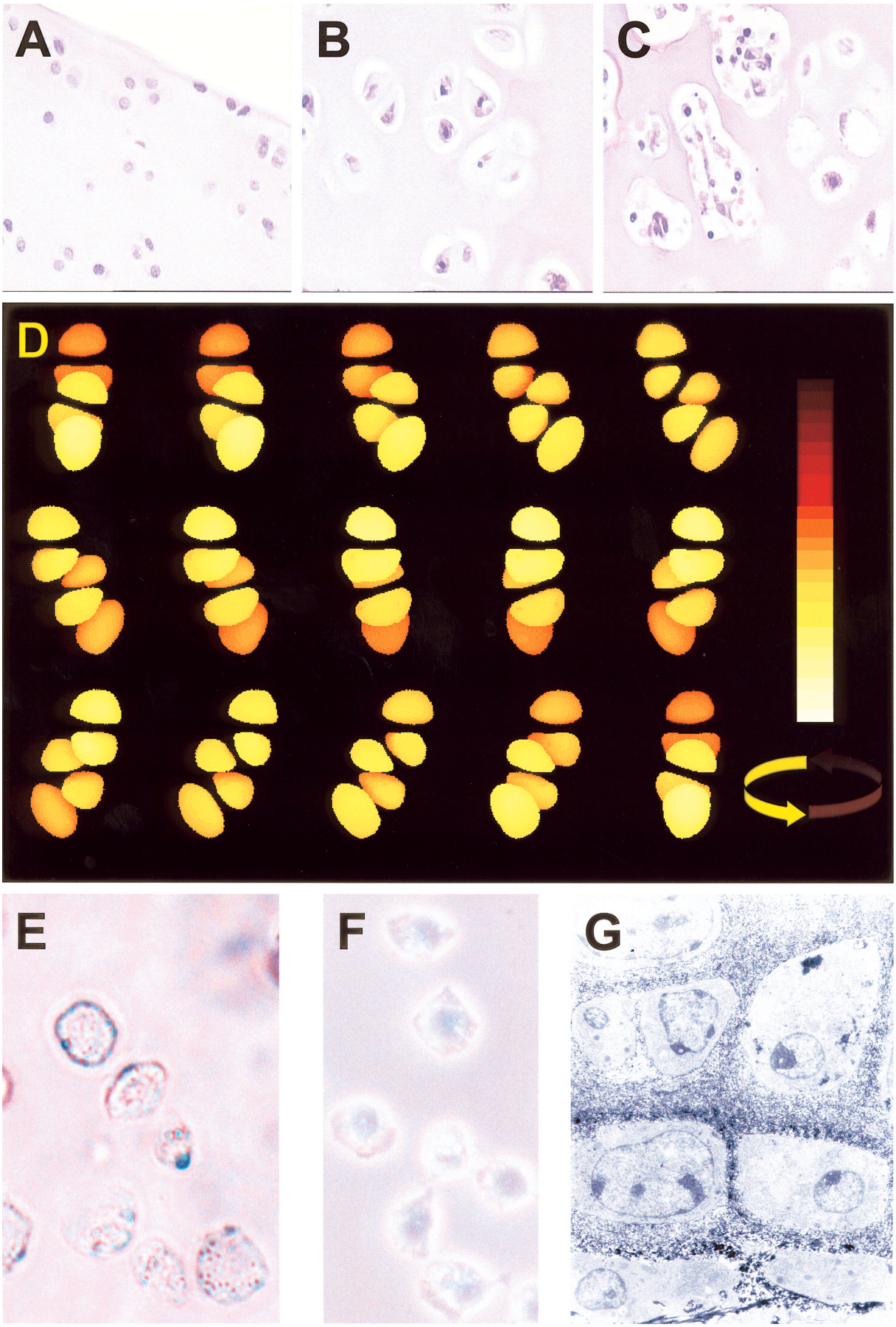
Healthy bones and joints depend on a functional endocrine system. It is a fact of clinical significance that excess IGF-I and growth hormone (GH) cause major joint pathology (Stavrou and Kleinberg, 2001). Endocrine disorders not only affect soft connective tissues but also implicate load-bearing musculoskeletal structures including bone, cartilage, synovium, tendon and ligament. Damage to soft connective tissue and associated innervation is a hallmark of acromegaly, hypothyroidism and diabetes mellitus (Lioté and Orcel, 2000). Acromegaly normally presents with quite severe arthritis involving degeneration of the spine and articular cartilage in peripheral joints (Stavrou and Kleinberg, 2001). Severe diabetes mellitus increases the risk of neuroarthropathy as a direct result of infection, neuropathy and vasculopathy. Pituitary tumours can have manifestations similar to rheumatological disease and may cause connective tissue disorders as a result of overproduction or deficiencies of pituitary hormones (Stavrou and Kleinberg, 2001). Excessive GH production by the pituitary gland causes cartilage destruction. GH deficiency on the other hand increases the risk of bone fractures.

Role of nutrients and regulators of nutrient metabolism

OA is a multifactorial, polygenic disorder; the pathogenesis of OA involves multiple aetiologies, including mechanical, biochemical, environmental, systemic and genetic factors that contribute to the imbalance in the synthesis and destruction of articular cartilage (Fig. 5A). Proinflammatory and catabolic cytokines (IL-1ß, TNF-α) are involved in disease initiation and progression. Equally, anabolic mediators such as insulin-like growth factor I (IGF-I), basic fibroblast growth factor (bFGF), connective tissue growth factor (CTGF) and transforming growth factor beta (TGFß) are responsible for stimulating articular chondrocyte matrix synthetic and mitotic activity, augment articular cartilage repair and inhibit chondrocyte mediated matrix catabolism (Pickart and Thaler, 1980; Trippel, 1995; Wozney, 1995; van den Berg, 1999; Haudenschild et al., 2001; Kumar et al., 2001; Yosimichi et al., 2001). However, these are intermediary effectors that can be triggered by a number of other factors.

One important contributing factor to the pathogenesis of OA is diet: nutritional deficiency and imbalance, obesity and diabetes (resulting in hypoglycaemia or hyperglycaemia) can result in metabolic and systemic disturbances that in turn increase susceptibility to OA directly due to effects on cartilage. Recent studies suggest that cartilage damage in OA occurs coincident with metabolic dysfunction, nutrient imbalance and diabetes mellitus (Rosenbloom and Silverstein, 1996; Denko and Malemud, 1999; Okma-Keulen and Hopman-Rock, 2001). Furthermore, high carbohydrate diets and generalized vitamin deficiency cause metabolic damage to cartilage (Willhelmi, 1993b). Vitamins E, B2, and C have been shown to exert an inhibitory effect on OA in animals. Chondrotoxic damage may result from food contaminants and fluoroquinolones (Stahlmann et al., 1995, 2000;

Fig. 3. Micrographs of porcine articular cartilage stained with hematoxylin and eosin clearly showing the zonal organization of cartilage and morphology of chondrocytes in the surface **(A)**, middle **(B)** and deep **(C)** zones. The confocal rotation series **(D)** illustrates a group of five chondrocytes from the middle zone of bovine metacarpal phalangeal cartilage. Isolated porcine chondrocytes maintain their morphology in suspension culture **(E)** but rapidly dedifferentiate in 2-dimensional culture and begin to take on fibroblastic characteristics **(F)**. The electron micrograph shown in panel G illustrates chondrocytes in 3-dimensional organoid culture isolated from the limb buds of 7-day-old mice. E, F, x 400; G, x 6,500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G

Shakibaei et al., 1996; Forster et al., 1998). Equally, mineral deficiency (i.e. calcium, magnesium, zinc, selenium and boron) can provoke skeletal damage in humans and animals.

Another joint disorder, which has a dietary component, is osteochondritis dissecans (OCD). In this disorder articular cartilage fragments separate from the articular surface and break off into the joint space (Williams et al., 1998). OCD in food-producing animals is caused by over-nutrition from excess protein and carbohydrate consumption and over supplementation (Slater et al., 1992). The overwhelming majority of musculoskeletal problems in companion animals and rapidly growing food-producing animal species are linked with a possible nutritional-related aetiology.

Therefore, it seems reasonable to suggest that a well-balanced, low fat and moderate carbohydrate diet together with nutritional supplements, vitamins and essential minerals will benefit individuals susceptible to degenerative joint disorders. Although nutrition alone will not be responsible for the pathogenesis of OCD or any of the developmental musculoskeletal diseases, it has been suggested that the development and progression of OCD and other orthopaedic diseases may be influenced by nutritional management (altering food and nutrient profile; Richardson and Zentek, 1998). Furthermore, chondroprotective nutraceutical supplements and modulation of nutrient transporters (Fig. 5B) may hold promise for developing future strategies for the prevention, treatment and cure of OA, OCD and related joint diseases. This approach may also be of benefit in treating soft connective tissue disorders in humans and animals.

Diffusion of nutrients in the ECM

Chondrocytes and the ECM are mutually dependent on each other. The matrix is not solely concerned with encapsulating the chondrocyte and protecting it from mechanical damage during joint loading. Many molecules pass through the matrix, including nutrients, substrates for the synthesis of matrix molecules, newly biosynthesised ECM macromolecules, matrix proteases, degraded matrix molecules, metabolic waste products, growth factors, hormones, and cytokines. In some instances these molecules may be stored in the matrix (Buckwalter and Mankin, 1998b). The types of molecules that pass through the matrix and the rate at which they can travel depends on the size and charge of the molecule, the composition and organisation of the

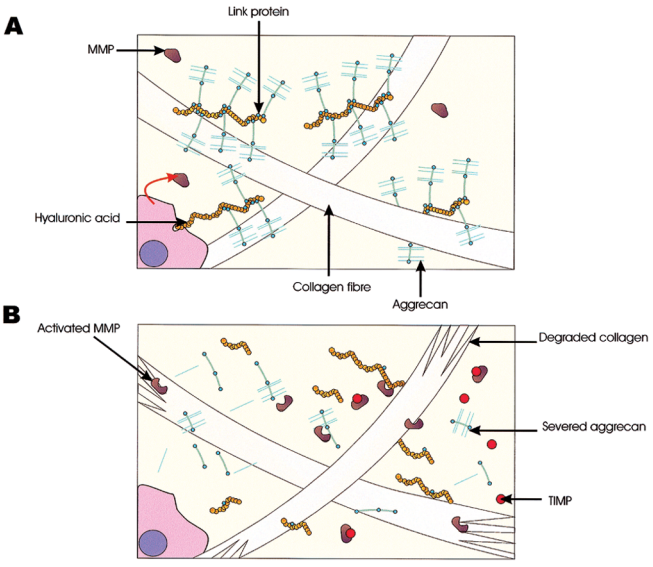

Fig. 4. A. In healthy cartilage the chondrocyte is surrounded by a matrix of collagen and hydrated proteoglycans, mainly aggrecan. Aggrecan is bound to long strands of hyaluronan via link proteins. **B.** In osteoarthritis there is a shift from the normal balance between matrix formation and destruction towards cleavage of matrix components. Aggrecan is primarily severed by aggrecanase and collagens are degraded by collagenases. The enzymes are collectively known as matrix metalloproteinases.

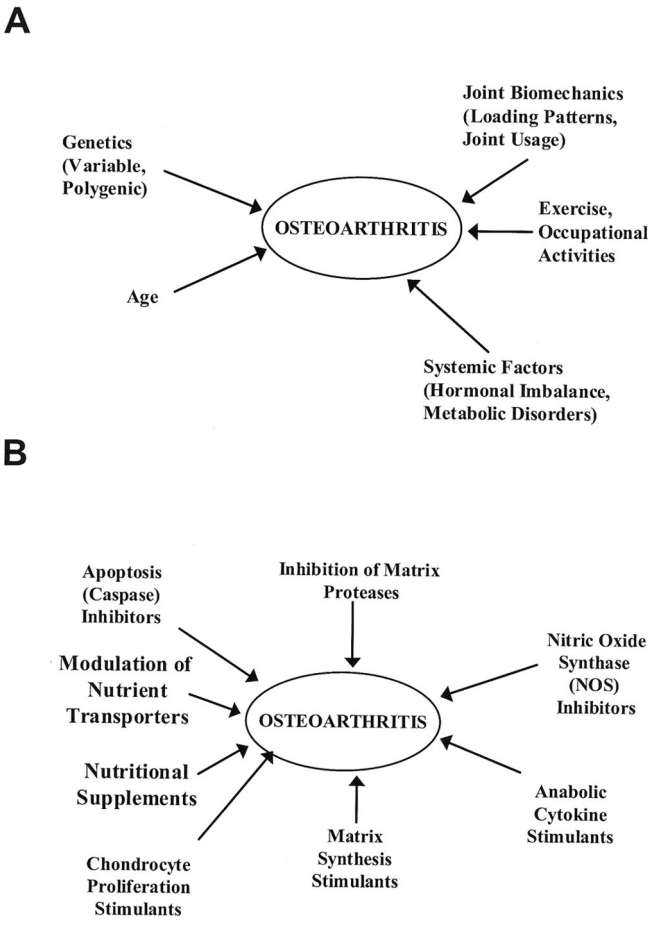

Fig. 5. A. Factors that pre-determine susceptibility to osteoarthritis. **B.** Rational therapies that may be used for the treatment of osteoarthritis and related connective tissue disorders.

matrix, particularly the concentration, composition and organization of large proteoglycans. Generally, monovalent and divalent ions, glucose, amino acids and water freely pass through the ECM (Fig. 6; Torzilli et al., 1997, 1998).

Provision of nutrients

How does cartilage obtain its nutrients? This has been a relatively controversial subject in connective tissue research and the answer to this question very much depends on which review article you read. The chondrocyte holds a key position in the maintenance of cartilage matrix and in repair responses during the development of OA. As the only living element of articular cartilage, the chondrocyte produces matrix components using nutrients provided by the surrounding tissues. In the course of its life, the chondrocyte is susceptible to nutritional deficiency and nutritional imbalance (excess quantities of certain nutrients and waste products). Perhaps before discussing the routes for nutrient provision we should clarify exactly which nutrients are needed by chondrocytes. The principal requisites are glucose, amino acids, vitamins and trace minerals, all essential for cartilage matrix synthesis and maintenance.

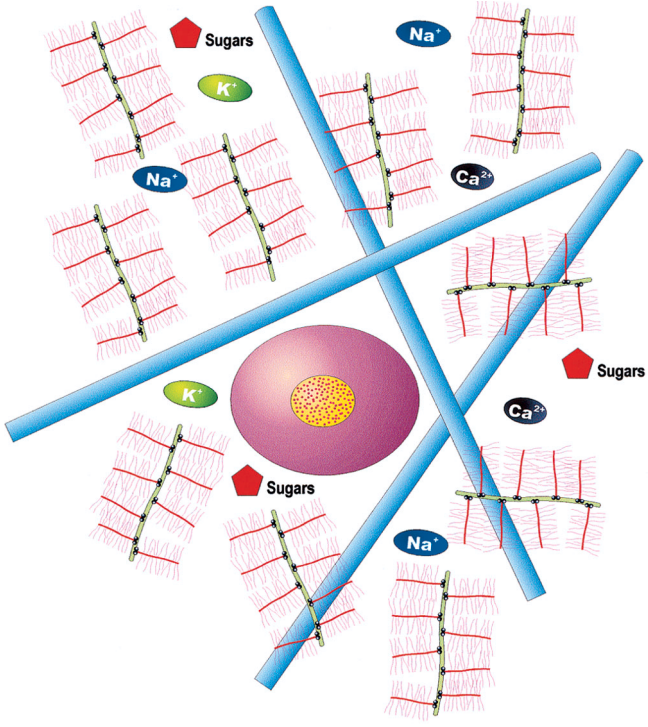

Fig. 6. Schematic model of an articular chondrocyte surrounded by a matrix of type II collagen and proteoglycans. Diffusion of large substances is impeded by the ECM but cations, gases and solutes such as sugars and amino acids freely diffuse and move within the matrix.

Cartilage canals as vascular channels in subchondral bone

Around the third foetal month of human development, vascular canals coming from the perichondrium are recognized in the mineralized epiphyseal cartilage. Thus, cartilage endplates contain conduits through which nutrients are provided to developing, immature articular cartilage (Burkus et al., 1993). These cartilage canals are important not only in the nutrition of the chondroepiphysis but also in the initial endochondral osteogenesis of the secondary ossification centre (Cole and Wezeman, 1985; Yamaguchi et al., 1990). The vascular supply through cartilage canals plays a critical role in developing sufficient biological inertia for the ossifying transition (Ganey et al., 1992). Cartilage canals develop through invasive fibroblast (of mesenchymal origin) and macrophage mediated chondrolysis (Chappard et al., 1986; Cole and Wezeman, 1987; Skawina et al., 1994). It has been suggested that growth of cartilage canals involves programmed cell death stimulated by thyroid hormone (Delgado-Baeza et al., 1992). Canals are generally believed to close before fully mature cartilage is formed. Furthermore, age-related changes have been observed in the arterioles, capillaries, and venules found in the nutrient canals adjacent to cartilage or intervertebral disc (Bernick and Cailliet, 1982). The calcification of the articular cartilage and vascular changes seen in the older vertebrae would be expected to impede the passage of nutrients from the blood to intervertebral disc cartilage.

Microcirculation within the joint

In human, rabbit and canine articular cartilage there are capillaries running through the subchondral bone in cylindrical channels 20-40 μm wide (Clark, 1990). These channels are surrounded by concentric lamellae of bone and only a minority of these channels open into the calcified articular cartilage. Most vascular channels are separated from the cartilage by a defined layer of bone. Hence it has been suggested that vessels within subchondral bone are present primarily to supply the bone with nutrients. The supply of chondrocytes is mediated mainly by the synovial microcirculation system. The microcirculation of the synovial joints is well adapted to its primary function which is to supply nutrients to the avascular cartilage, whose chondrocytes are metabolically active but are relatively large distances from the nearest blood capillary; the nutrients involved may have to diffuse over 1 cm to access chondrocytes in the centre of a human knee (Levick, 1995). Material exchange is facilitated by a high density of fenestrated capillaries situated very close to the synovial surface with fenestrations preferentially oriented toward the joint cavity. Even so, diffusion alone is too slow to supply central chondrocytes with glucose. The problem is solved by the synovial microcirculation generating intra-

articular fluid (synovial fluid) that transports glucose and other nutrients during dynamic joint loading and physical exercise.

Effects of diet on cartilage canal development

Diet has an important effect on the development of cartilage canals (Woodard et al., 1987a). In an experimental model using piglets, diet has been shown to affect the development of cartilage and its subchondral canals. Piglets fed a reduced protein diet compared to control piglets have been shown to have reduced body weights, reduced longitudinal bone growth and evidence of early changes associated with OCD (Woodard et al., 1987b). Animals fed a low protein diet showed disorderly foci of endochondral ossification beneath their articulating cartilages (characterized by an area of chondrocyte necrosis preventing normal cartilage matrix mineralization). These abnormalities have been associated with abnormalities of the cartilage canal vessels, and chondrocyte necrosis was considered to precede degenerative changes in articular cartilage matrix (Woodard et al., 1987b).

IGF-I resistance implicates metabolic dysfunction in OA

Degenerative diseases of load bearing connective tissues such as cartilage and the intervertebral disc are generally characterized by disequilibria between ECM repair and degradative processes, with the former not keeping pace, resulting in a loss of proteoglycans, type II collagen, and other ECM components as a result of elevated matrix metalloprotease and aggrecanase activity (Buckwalter and Mankin, 1998c). It is becoming increasingly apparent that a number of degenerative conditions in cartilage occur coincident with endocrine diseases associated with metabolic dysfunction and glucose imbalance (Denko et al., 1994; Denko and Malemud, 1999). Independent reports indicate that the reduced growth and repair observed in degenerative cartilage disorders such as OA may be related to an inability of insulin-like growth factor I (IGF-I) to exert its physiological and anabolic effect on chondrocytes. In healthy cartilage IGF-I promotes differentiated cellular functions, which include stimulation of proteoglycan (PG), type-II collagen, and other ECM components. There is an age-related decline in the chondrocyte response to IGF-I and chondrocytes from human OA joints do not respond well to IGF-I stimulation (Doré et al., 1994). This anomaly known as the "IGF-I-resistant state" implicates insulin/IGF-I signalling and glucose metabolism in the pathogenesis of OA (Kelley et al., 1999).

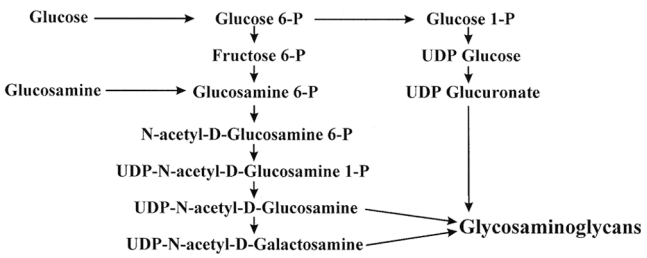

Fig. 7. The biochemical pathway for the synthesis of glycosaminoglycans from glucose and glucosamine as primary substrates.

Glucose: A metabolite and structural precursor for cartilage

Glucose is an important metabolite for all living cells and with other simple sugars and related molecules derived from these sugars provide sources of readily available energy for cells. Sugars also provide carbon skeletons for the biosynthesis of other macromolecules such as proteins, lipids, nucleic acids and complex polysaccharides (glycogen). Furthermore, hexose sugars are building blocks of glycoproteins such as proteoglycans. In addition to their role as structural components of the ECM (Fig. 7), proteoglycans fulfil adhesive and informational functions. Glucose has been shown to be an important metabolite and structural precursor for cartilage and its regular provision and uptake will have significant consequences for the development and functional integrity of the tissue (Wang et al., 1999). Glucose must diffuse across the synovium and the ECM before reaching chondrocytes (Torzilli et al., 1997). Chondrocytes are highly glycolytic cells and require a regular supply of glucose for optimal ATP production and cell homeostasis (Otte, 1991; Lee and Urban, 1997). Even modest changes in glucose concentrations in the extracellular microenvironment of chondrocytes, (such as those that can occur in poorly treated insulin-dependent diabetes mellitus (IDDM)), could impair IGF-I-mediated anabolic activities and thus promote a variety of joint pathologies (Rosenbloom and Silverstein, 1996; Lioté and Orcel, 2000). Therefore, the steady supply and transport of physiological levels of glucose is critical for chondrocyte viability and matrix synthesis.

Glucose metabolism in cartilage

Typical mammalian cells generate energy in the form of ATP by anaerobic or aerobic metabolism of glucose. In articular cartilage, the chondrocyte obtains glucose and oxygen by diffusion from the synovial fluid. The importance of chondrocyte glucose transport and metabolism for the synthesis of a normal, mechanically competent ECM is emphasized in Fig. 8. The concentrations of glucose and oxygen within cartilage matrix gradually diminish with increasing proximity to the calcified cartilage layer and this is particularly relevant in mature cartilage. The gradients for glucose and oxygen depend upon chondrocyte density and metabolic consumption of the cells. The gradient of the

partial pressure of oxygen (PO_2) provides the conditions for a negative Pasteur effect in chondrocytes (Lee and Urban, 1997). Anoxia severely inhibits glucose uptake and lactate production in cartilage; the decrease in lactate formation correlates well with decreased glucose uptake by chondrocytes and this reduction in the rate of glycolysis in anoxic conditions is seen as evidence for a "negative Pasteur effect" in cartilage (Lee and Urban, 1997). Conversely, in the intervertebral disc, anoxia and addition of glycolysis inhibitors result in a progressive positive Pasteur effect suggesting that unlike articular cartilage, a large proportion of the intervertebral disc's energy derives from oxidative phosphorylation (Ishihara and Urban, 1999). Manometric (Warburg technique) studies of porcine femoral head cartilage clearly show a close correlation between oxygen consumption and the concentration and consumption of glucose (the Crabtree effect; Otte, 1991). Excess glucose is channelled into synthetic and storage processes consistent with the physiological (ECM synthesis) role of chondrocytes in cartilage matrix (Otte, 1991). Under physiological conditions, glucose in the synovium reduces the consumption of oxygen in the well-nourished superficial layer of articular cartilage, thus allowing an oxidative compensation for the diminishing glycolysis rates in the deep zone. Thus the Crabtree effect has an important regulatory role in the basic metabolism of articular cartilage.

The GLUT/SLC2A family of hexose transporters

A family of highly homologous, integral membrane transport proteins, the GLUT/SLC2A family, catalyses the entry of sugars such as glucose into mammalian cells. These proteins are characterized by the presence of 12 membrane spanning helices and several conserved sequence motifs (Joost and Thorens, 2001). The GLUT/SLC2A proteins are expressed in a tissue- and cell-specific manner and exhibit distinct kinetic and regulatory properties that reflect their functional and tissue specific roles (Table 1). Over a dozen GLUT family members have been identified on the basis of sequence similarity (Joost and Thorens, 2001). The very recent discovery of the new GLUT/SLC2A members

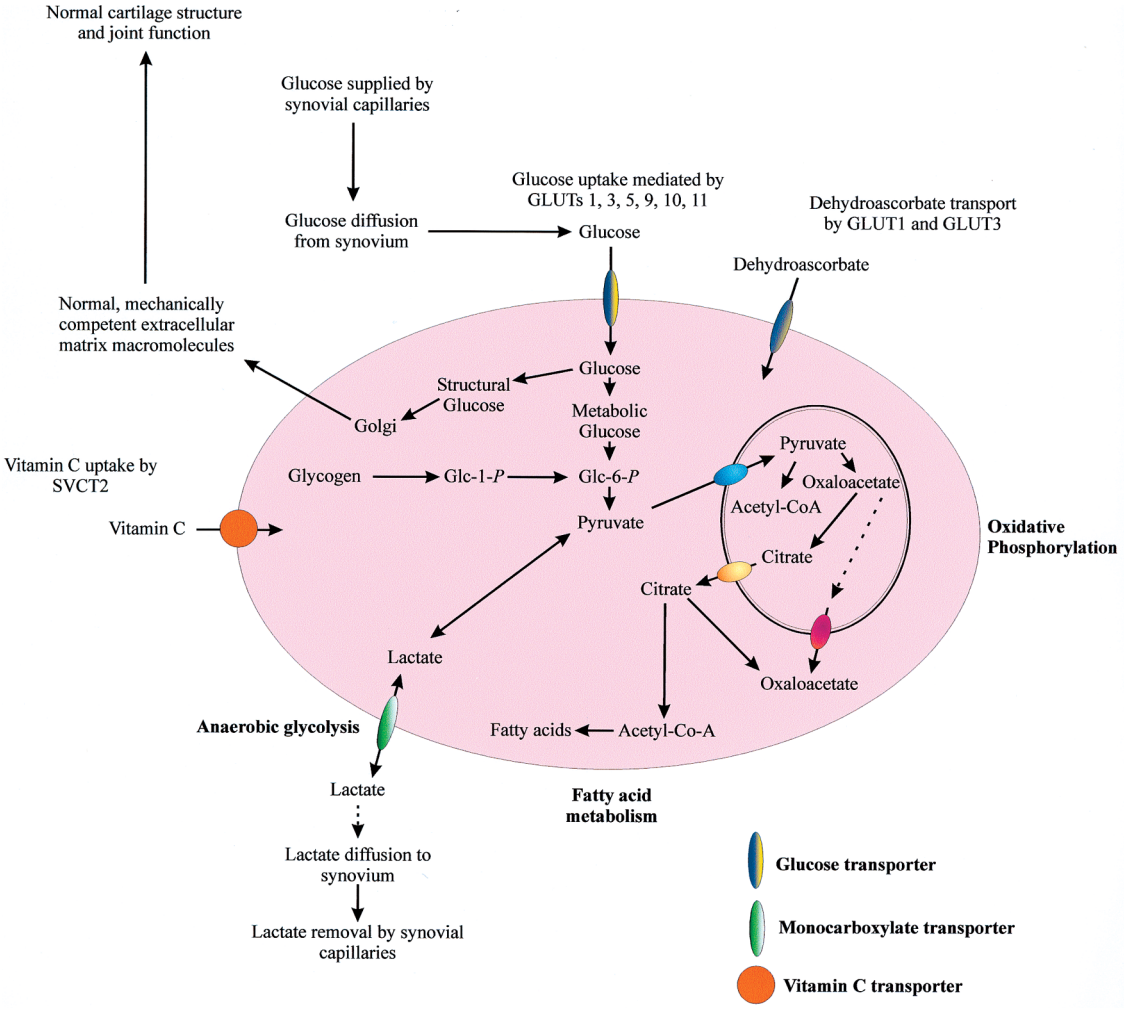

Fig. 8. Model of glucose diffusion and transport in articular cartilage. Glucose is delivered to the chondrocyte via the synovial microcirculation and take up by GLUT proteins. Intracellular glucose is accumulated in two distinct pools; the metabolic pool used for glycolysis and the structural pool used for synthesis of ECM macromolecules.

Table 1. Summary of the extended GLUT family including gene names from recently revised GLUT nomenclature proposed by Joost and Thorens 2001, cDNA accession numbers, proposed physiological function, limited tissue expression and relevant references.

PROTEIN	GENE NAME	cDNA	EXPRESSION	PROPOSED PHYSIOLOGICAL FUNCTION	REFERENCES
GLUT1	SLC2A1	K03195	Erythrocytes, brain (vascular), chondrocytes	Housekeeping glucose transporter; ubiquitously expressed; basal glucose uptake for growth and development; transport across red cells, neuronal cells (blood brain barrier) and other tissues. GLUT1 expression appears to be up-regulated by glucose deprivation and prolonged hypoxia-ischemia. Expressed in almost all cultured cells	Mueckler et al., 1985; Shikhman et al., 2001b; Neama et al., 2001; Mobasheri et al., 2001, 2002a Hamrahian et al., 1999; Behrooz and Ismail-Beigi, 1997; Vannucci et al., 1996, 1998; Zhang et al., 1999
GLUT2	SLC2A2	J03810	Liver, pancreatic ß cells, intestine	High-capacity, low affinity transporter expressed in the basolateral membrane of hepatocytes, pancreatic ß cells and enterocytes	Fukumoto et al., 1988; Thomson and Wild, 1997a/b; Schuit, 1996; Bell et al., 1990
GLUT3	SLC2A3	J04069	Brain (neurons and glia), chondrocytes	Fast glucose transporter; Basal transport of glucose into many human cells; responsible for glucose uptake from cerebral fluid into parenchymal neurons; high affinity transporter specialized for glucose uptake where substrate concentrations are low	Kayano et al., 1988; Vannucci and Vannucci, 2000; Behrooz and Ismail-Beigi, 1997; Vannucci et al., 1996, 1998 Shikhman et al., 2001b; Neama et al., 2001; Mobasheri et al., 2001, 2002a,c
GLUT4	SLC2A4	M20747	Muscle, fat, heart, chondroblasts, growth plate chondrocytes	Insulin sensitive glucose transporter responsible for rapid glucose transport following insulin stimulation; implicated in diabetes	Fukumoto et al., 1989; Vannucci et al., 2000; Wang et al., 1999; Mueckler, 1994; Ohara et al., 2001
GLUT5	SLC2A5	J05461	Intestine, testis, kidney, chondrocytes; lower levels in other tissues	Fructose transporter expressed in the intestine; responsible for transport of fructose and other hexoses	Kayano et al., 1990; Ohara et al., 2001; Mobasheri et al., 2002c
GLUT6 (alias GLUT9)	SLC2A6	Y17803	Spleen, leukocytes, brain	Regulated by dynamin-dependent recycling between the plasma membrane and internal membranes	Doege et al., 2000a; Joost and Thorens, 2001; Dawson et al., 2001; Lisinski et al., 2001
GLUT7	SLC2A7	cDNA not yet cloned	Unknown	Unknown	
GLUT8 (alias GLUTX1)	SLC2A8	Y17801	Testis, blastocyst, brain and other insulin-regulated tissues	Regulated by dynamin-dependent recycling between the plasma membrane and internal membranes; involved in pre-implantation development with other glucose transporters (GLUT1, GLUT3, GLUT5 and SGLT1). GLUT8 expression appears to be down-regulated by glucose deprivation and prolonged hypoxia	Doege et al., 2000b; Carayannopoulos et al., 2000; Ibberson et al., 2000; Dawson et al., 2001; Scheepers et al., 2001
GLUT9 (alias GLUTX)	SLC2A9	AF210317	Kidney, liver, chondrocytes	Glucose transporter, expressed in kidney, liver and chondrocytes	Phay et al., 2000; Shikhman et al., 2001b; Mobasheri et al., 2002a,
GLUT10	SLC2A10	AF321240	Heart, lung, liver, pancreas, skeletal muscle, placenta, kidney, chondrocytes	Low K_m (0.3 mM) glucose/galactose transporter; implicated in glucose metabolism and type 2 diabetes	McVie-Wylie et al., 2001; Mobasheri et al., 2002c; Dawson et al., 2001
GLUT11 (alias GLUT10)	SLC2A11	AJ271290	Heart, skeletal muscle, chondrocytes	Glucose transporter with several transcription variants (products of mRNA splicing)	Doege et al., 2001a, b; Mobasheri et al., 2002c; Sasaki et al., 2001
GLUT12 (alias GLUT8)	SLC2A12	AY046419	Heart, skeletal muscle, prostate	Expressed in skeletal muscle, adipose tissue, and small intestine; GLUT-12 may comprise a second insulin-sensitive glucose transport system.	Rogers et al., 1998, 2002
HMIT	SLC2A13	AJ315644	Brain	Proton/myo-inositol symporter expressed predominantly in the brain; expression in other tissues not well studied	Uldry et al., 2001

combined with the previously heterogeneous and often confusing nomenclature has led to extensive revision and introduction of a systematic nomenclature (Joost et al., 2002). The sequence similarities of the GLUT/SLC2A family members have been defined (Fig. 9), and the family now divided into three subclasses. Class I contains the first identified, fully characterized glucose transporters GLUT1-4, distinguishable by their tissue distribution, kinetic properties, and hormonal regulation. GLUT1 is found in most tissues, but is especially abundant in erythrocytes, and blood-tissue endothelial and epithelial borders, such as the blood-brain barrier, retina, and placenta. GLUT2 is a low affinity, high K_m isoform expressed in the liver, gut and pancreatic islets (Mueckler, 1994; Thorens, 2001). GLUT3 is primarily expressed in brain where it is localized exclusively to neurons; non-neuronal sites of expression include placenta, sperm, and human platelets (Shepherd et al., 1992; Haber et al., 1993; Burant and Davidson, 1994; Craik et al., 1995). GLUT3, like GLUT1, is a high-affinity transporter but differs from GLUT1 in the rate at which it transports glucose; its turnover number is seven times higher than GLUT1 (Maher et al., 1996) making GLUT3 one of the fastest glucose transporters (see later). GLUT4 is an "insulin-responsive" isoform expressed in skeletal muscle, heart, white and brown adipose tissue, tissues which respond to insulin or contraction by acutely increasing their rates of glucose uptake. Class II consists of the fructose transporter GLUT5 and three related but as yet poorly characterized proteins, GLUT7, GLUT9 and GLUT11. Class III GLUT/SLC2A members are characterized by the lack of a glycosylation site in the extracellular loop 1, and by the presence of such a site in loop 9. The recently cloned proton-myoinositol symporter (HMIT; Uldry et al., 2001) has been included in class III, despite the fact that it does not transport glucose but is a polyol transporter. Functional glucose transport has been demonstrated for GLUT6 (formerly GLUT9) and GLUT8 (formerly GLUTX1). It must be emphasized that the designation of the family as "glucose transporters" does not reflect the substrate specificity of individual members, which may transport hexose sugars or polyols other than glucose (e.g. GLUT5 transports fructose; HMIT1 transports myoinositol, other GLUTs may transport sugar-derived compounds).

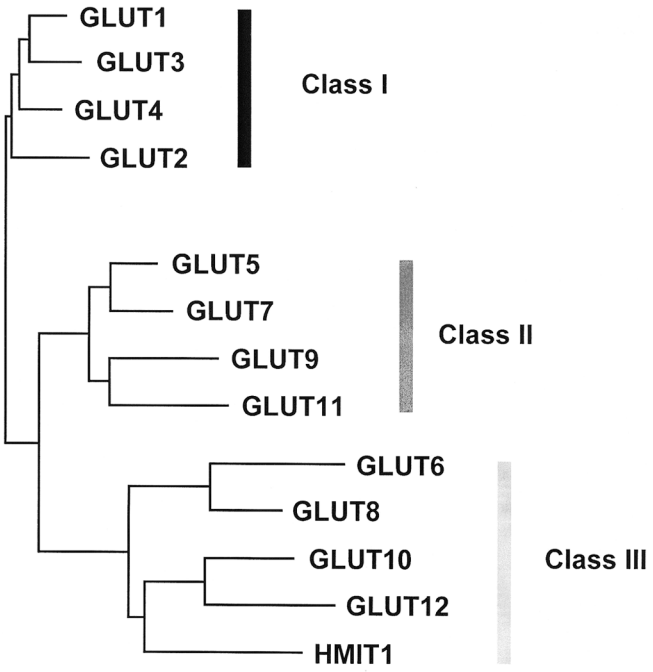

Fig. 9. Dendrogram of a multiple alignment of all members of the extended GLUT family. Chondrocytes may express up to three members of class I and class II GLUTs and at least one member of class III. Reproduced from Joost and Thorens (2001) with permission of Taylor and Francis Ltd.

Role of GLUT4 mediated glucose transport in the developing mesenchyme

Recent studies by Vannucci and co-workers (2000) suggest that the insulin-responsive facilitative glucose transporter GLUT4 is involved in embryonic mesenchymal development in the mouse. GLUT4 mRNA and protein expression is evident in the

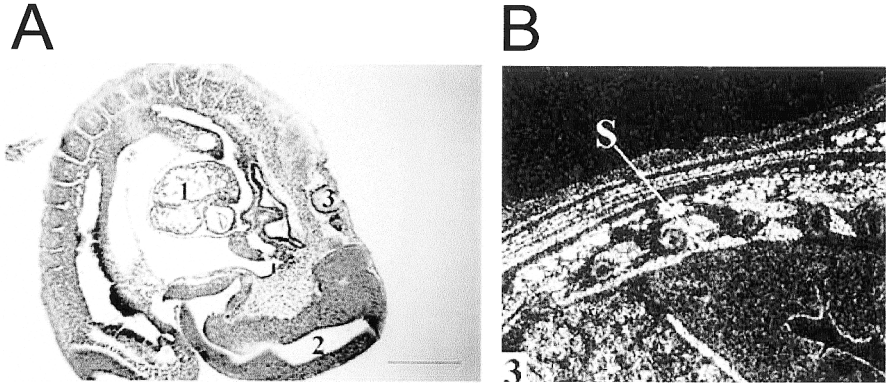

Fig. 10. GLUT4 expression in the developing mouse embryo. **Panel A** shows immunohistochemical analysis of GLUT4 protein expression in the E10 mouse embryo. Intense GLUT4 immunoreactivity is present throughout the embryo, including the craniofacial mesenchyme, cartilage and bone. **Panel B** is a dark-field micrograph of emulsion-dipped sagittal sections for the whole fetus showing GLUT4 mRNA expression in the spine of the E18 mouse embryo (Reproduced from Vannucci et al., 2000, with permission of S.J. Vannucci and S. Karger AG). Bar: 500 nm.

craniofacial mesenchyme and in cartilage and bone during development and throughout early postnatal life (Fig. 10). Postnatally, GLUT4 is primarily expressed in insulin-sensitive tissues, i.e. adipose tissue, heart and skeletal muscle, where it is localised to an intracellular membrane compartment in the basal, or unstimulated state. These tissues respond to either insulin or contraction by promoting a rapid translocation of intracellular GLUT4 to the plasma membrane thereby acutely increasing glucose uptake (Rea and James, 1997; James et al., 1989; Marette et al., 1992, 1999; Czech and Corvera, 1999; Pessin et al., 1999). The presence of GLUT4 in bone and cartilage in early stages of development suggests that this transporter isoform is involved in chondrogenesis, possibly also through stimulation of a similar translocation mechanism. The signal transduction pathway involved in insulin stimulation of GLUT4 is initiated by the stimulation of the insulin receptor tyrosine kinase, which, leads to the phosphorylation of insulin receptor substrate (IRS), the activation of PI-3 kinase and stimulation of Akt/PKB. It is this step that promotes the translocation of the GLUT4-containing vesicles from the intracellular compartment to the plasma membrane (Holman and Kasuga, 1997; Avruch, 1998). Growth factor stimulation of glucose uptake during chondrogenesis may well involve a similar pathway through Akt/PKB activation (see below). Furthermore, expression of GLUT4 in chondroblasts and chondrocytes during development might be regulated by insulin and IGF-I, as it is in adipocytes and skeletal muscle cells. The fact that skeletal muscle, adipose tissue and cartilage all respond to IGF-I may be due to their shared mesenchymal origin (Grigoriadis et al., 1990). Therefore, it is possible that IGF-I and IGF-II could regulate GLUT4 through engagement of the insulin or IGF-I receptors (Vannucci et al., 2000).

Regulation of glucose and glycogen metabolism in the growth plate

Insulin-like growth factors I and II (IGF-I, IGF-II) affect the growth and carbohydrate metabolism of differentiating cartilage. IGF-II stimulates glucose uptake by chondrocytes at various stages of differentiation and thus can be considered as a regulator of glucose transport and metabolism in developing cartilage (Bhaumick and Bala, 1991). Glycogen stores are normally accumulated by proliferative and early hypertrophic chondrocytes and depleted during maturation of the hypertrophic chondrocytes (Pritcherd, 1952; Brighton et al., 1969). A well-known target of insulin action is the enzyme glycogen synthase kinase 3ß (GSK3ß). As discussed above, activation of the insulin receptor initiates a signalling cascade proceeding through PI3K and Akt/PKB and results in the serine phosphorylation and inhibition of GSK3ß activity (Cohen et al., 1997). This in turn relieves GSK3ß-induced inhibition of glycogen synthase, thus promoting glycogen synthesis. GSK3ß immunoreactivity is abundant in proliferative and hypertrophic chondrocytes in the epiphyseal growth plate.

The factors involved in regulating the rapid expansion of hypertrophic chondrocytes have remained unknown. Recent data published by Wang and co-workers (1999) suggest that IGF-I enhances chondrocyte hypertrophy by insulin-like actions such as boosting glucose and amino acid uptake and utilization. Supporting this view, the IGF-I receptor, which is structurally and functionally homologous to the insulin receptor, is known to mediate such anabolic effects (De Meyts et al., 1994), including regulation of GLUT4 (Wilson et al., 1995). GLUT4 expression and GSK3ß serine phosphorylation are significantly diminished in IGF-I null hypertrophic chondrocytes, resulting in reduced glycogen in these cells (Fig. 11). Hypertrophic chondrocytes are highly active metabolically and dependent upon glycolysis to fuel their expansive biosynthetic activity (Brighton et al., 1983). The decrease in rRNA in IGF-I null hypertrophic chondrocytes may reflect cellular ‘starvation’ for fuel and building blocks for protein synthesis. IGF-I probably acts in an insulin-like manner to enhance chondrocyte glucose uptake and glycogen and protein synthesis, thus promoting maximal somatic growth and matrix production and enhancing the rate of linear bone growth. These studies and recent observations by Ohara et al. (2001) highlight the importance of glucose for cartilage metabolism and long bone growth. We speculate that glucose and polyol transporters together with aquaporins and other volume regulatory systems are involved in uptake of osmolytes and water to increase the osmotic pressure within hypertrophic chondrocytes resulting in an extraordinary increase in cell size and volume characteristic of hypertrophy.

GLUT expression correlates with glucose consumption in the growth plate

The study by Ohara et al. (2001) suggests that GLUTs are present in the epiphyseal growth plate, particularly in the hypertrophic chondrocytes and that their expression correlates with metabolic activity (determined by using ^{14}C-2-deoxyglucose autoradiography). Their data shows that GLUT expression is particularly strong in the maturation zone of growth plates in juvenile rats. These observations could be partially explained by considering the vascular supply of the growth plate via the epiphyseal artery (Iannotti, 1990). Growth plate chondrocytes receive significantly more glucose and oxygen compared to articular chondrocytes. Therefore, epiphyseal chondrocytes are significantly better nourished compared to articular chondrocytes.

Zonal differences in chondrocyte GLUT expression in epiphyseal cartilage may be related to demand for metabolic substrate. In the growth plate, chondrocytes undergo rapid cell division in the proliferating zone

concomitant with massive changes in cell size and volume in the hypertrophic zone. Requirements for metabolic and structural glucose will be different in these two zones. This is particularly important in the hypertrophic zone where large amounts of glycogen are stored but where glycolysis levels are low (Brighton et al., 1973). Expression of GLUTs in the proliferative zone is lower than the hypertrophic zone supporting the hypothesis that maturing and hypertrophic chondrocytes require large quantities of glucose for metabolic activities and glycogen storage.

Ohara and co-workers (2001) also examined GLUT1-5 mRNA expression in epiphyseal chondrocytes from juvenile rats by RT-PCR and found transcripts encoding GLUT1, GLUT2, GLUT3 and GLUT5, but they did not detect GLUT4 at this developmental stage compared to earlier developmental stages. These observations and the studies by Wang et al. (1999) and Vannucci et al. (2000) suggest that GLUT4 expression in cartilage is developmentally regulated and may be influenced by insulin and IGF-I. These studies, in combination with our recent work on mature cartilage (Mobasheri et al., 2001, 2002a; Neama et al., 2001), and the observations of Shikhman and colleagues (2001b) collectively enforce the notion that GLUT4 does not play a physiological role in mature cartilage where its expression is lost or significantly down-regulated (Shikhman et al., 2001b).

The expression of GLUT5 in rat epiphyseal chondrocytes (Ohara et al., 2001) and human articular chondrocytes (Mobasheri et al., 2002c) suggests that fructose may also be used an energy source or structural sugar. However, since the substrate specificity and transport kinetics of GLUT5 are not well studied, it may be likely that GLUT5 or indeed other GLUTs are responsible for uptake of related pentose and hexose derived structures that serve as precursors for the synthesis of sulfated matrix glycosaminoglycans. However, GLUT5 is a very poor transporter for glucose; a caveat to GLUT5 expression is its localization to cells of macrophage/monocyte lineage. Thus it may be possible that GLUT5 is not actually expressed in chondrocytes but is expressed by monocytes in the subchondral bone and growth plate. Further studies are required to confirm expression of GLUT5 in cartilage and investigate its functional role.

Vitamin C: A simple sugar essential for matrix synthesis

Vitamin C (ascorbic acid) is a hexose derivative and powerful reducing agent synthesized from glucose. Enzymatic reactions that require vitamin C include those involved in the synthesis of hydroxyproline and hydroxylysine. Vitamin C acts as an enzyme cofactor and antioxidant that stimulates the transcription, translation, and post-translational processing of collagen in connective tissue cells (Francheschi, 1992). Despite the importance of vitamin C for connective tissue turnover, there is limited published information about its transport and metabolism in chondrocytes and osteoblasts.

Osteoblasts share a number of phenotypic characteristics with chondrocytes and like chondrocytes they are derived from mesenchymal stem cells (Grigoriadis et al., 1990). In osteoblasts a saturable, specific and stereo-selective (low uptake for D-isoascorbic acid) transport system exists for vitamin C. The maximum capacity of this Na^+-dependent vitamin C transporter is within the range of normal serum levels of vitamin C (10-100 μM/L) (Wolf, 1996). Vitamin C increases hydroxylation (by prolyl- and lysyl-hydroxylases) of pro-collagen approximately 5 fold (Francheschi and Iyer, 1992). This is an important stimulus for increased transcription of pro-collagen mRNA, translation and expression of pro-collagen protein and ultimately critical for ECM synthesis and turnover. In osteoblasts, ascorbic acid influences osteoblast differentiation and is a co-factor for collagen synthesis and deposition as well as mineralization (Bellows et al., 1986; Sugimoto et al., 1986; Franceschi et al., 1995). There are specific L-ascorbate transporters in the plasma membrane of calvarial cells and osteoblastic cell lines (Dixon and Wilson, 1992; Franceschi et al., 1995; Wilson and Dixon, 1995). Insulin also modulates the differentiation and synthetic activity of osteoblasts and studies show that rat osteoblast-like cells (clone UMR-106) accumulate ascorbic acid intracellularly when incubated with dehydroascorbic acid (DHAA; oxidized form of vitamin C) (Qutob et al., 1998). Moreover, insulin apparently increases the intracellular concentration of ascorbic acid derived from DHAA and also increases the initial rates of uptake of DHAA and 2-deoxyglucose, but not that of ascorbate itself.

Ascorbic acid has long been associated with the slowing of OA in guinea pigs (Schwartz et al., 1981; Meacock et al., 1990). It has also been known for some time that there are specific vitamin C transporters, which accumulate vitamin C in chondrocytes and osteoblasts. Clark and co-workers (2002) have recently studied the expression of the newly described vitamin C transporters (SVCTs; Tsukaguchi et al., 1999) in guinea pig chondrocytes where they identified transcripts for SVCT2 but not SVCT1 by touchdown RT-PCR, sequencing and homology comparison to existing sequences in GenBank. They also found that synthesis of type II collagen, prolyl 4-hydroxylase and aggrecan increased in chondrocytes exposed to various antiscorbutic forms of ascorbic acid (i.e. L-ascorbate and ascorbate-2-phosphate). Their data represents the first-ever molecular characterization of ascorbate transport and metabolism and suggests that SVCT2 is the primary transport mechanism by which L-forms of ascorbate enter the chondrocyte to bring about changes in the transcription and translation of matrix genes. SVCT2 is expressed in most tissues except lung and skeletal muscle (Liang et al., 2001).

Bone growth and remodelling are decreased in insulin-dependent (type I) diabetes mellitus, leading to osteopenia and osteoporosis (Boullion, 1990; Munoz-Torres, 1996). This suggests that excess plasma/synovial glucose has detrimental effects on matrix synthesis and calcification. On the other hand, animals with hyperinsulinemia, have increased bone mineral density (Verhaeghe et al., 1994a,b). In osteoblasts vitamin C stimulates osteoblast-mediated production of osteocalcin, alkaline phosphatase and bone matrix synthesis. In chondrocytes vitamin C plays an important role in the regulation of genes encoding matrix macromolecules and calcification in chondrocytes within calcified cartilage and the deep zones of articular cartilage. Further studies are needed to determine the pharmacological characteristics of Na^+ dependent vitamin C transporters (SVCTs) in articular chondrocytes.

GLUTs: candidates for the transport of glucose, glucosamine and vitamin C

The oxidized form of vitamin C (dehydroascorbic acid) is transported by facilitative glucose transporters GLUT1, GLUT3 and GLUT4 in a variety of cell models (Vera et al., 1995; Wolf, 1996; Agus et al., 1997, 1999; Rumsey et al., 1997, 1999; Rumsey et al., 2000; Vera et al., 2001). Thus, dehydroascorbic acid uptake may also occur via GLUT 1, 3 and 4 in chondrocytes in addition to SVCT2. However, under physiological conditions these transporters are unlikely to play a major role in the uptake of vitamin C as high concentrations of glucose will effectively block such an uptake and dedicated vitamin C transporters (i.e. SVCT2) are required to take advantage of the normally steep inward Na^+ gradient into chondrocytes and transport ascorbate (Liang et al., 2001). There is also some evidence to suggest that GLUTs participate in the transport of glucosamine and N-acetylglucosamine (Rauchman et al., 1992; Cloherty et al., 1995), which can reduce the inflammatory responses of chondrocytes (Shikhman et al., 2001a) and serve as efficient precursors of glycosaminoglycan synthesis (Mason et al., 1982).

Evidence for up to six GLUT isoforms in mature human chondrocytes

The expression of GLUT/SLC2A family members has not been extensively studied in normal or diseased articular cartilage. We have molecular evidence for the expression of at least six members of the GLUT/SLC2A family in human articular chondrocytes (Mobasheri et al., 2002a,c; Fig. 11A). These include GLUT1, GLUT3, GLUT5, GLUT9, GLUT10 and GLUT11.

We have shown that human articular chondrocytes isolated from fully developed normal cartilage contain mRNA for GLUT1, GLUT3 and GLUT9 (Mobasheri et al., 2002a). We have purified, cloned and sequenced the PCR products corresponding to GLUTs 1, 3 and 9 to confirm expression of these GLUTs in human chondrocytes. The GLUT1 sequence obtained was 100% identical to sequences already deposited in GenBank corresponding to the *Homo sapiens* facilitated glucose transporter, member 1, GLUT1 (SLC2A1). The partial sequence was deposited in GenBank under accession number AY034633. The GLUT3 sequence we obtained

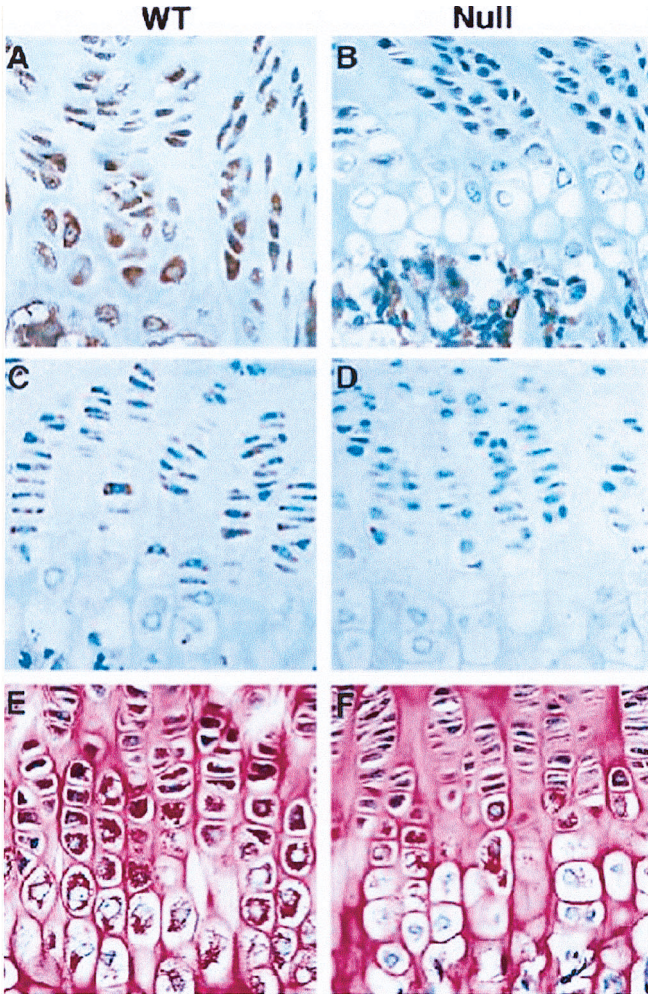

Fig. 11. Evidence for expression of GLUT4 and reduced metabolic function in IGF-I null chondrocytes compared to wild type chondrocytes. **Panels A, C and E** are representative sections of wild-type growth plate. Panels **B, D and F** are sections of IGF-I null growth plate. Immunostaining of the GLUT 4 glucose transporter is shown in **A and B;** very little GLUT 4 is detected in IGF-I null chondrocytes but is abundant in bone marrow stromal cells (as indicated by arrows in **panel B)**. Immunodetection of the inactive form enzyme glycogen synthase kinase (GSK3ß) an important target of insulin action responsible for glycogen metabolism is also reduced in IGF-I null mice. PAS histochemical staining for glycogen also shows significantly reduced glycogen stores in IGF-I null growth plate chondrocytes **(F)** compared to wild type growth plate chondrocytes **(E)**. Reproduced from Wang et al., FASEB J., 1999 vol. 13, 1985-1990 with kind permission of C.A. Bondy (Developmental Endocrinology Branch, NICHD, National Institutes of Health, Bethesda, U.S.A.) and the Federation of American Societies for Experimental Biology (FASEB).

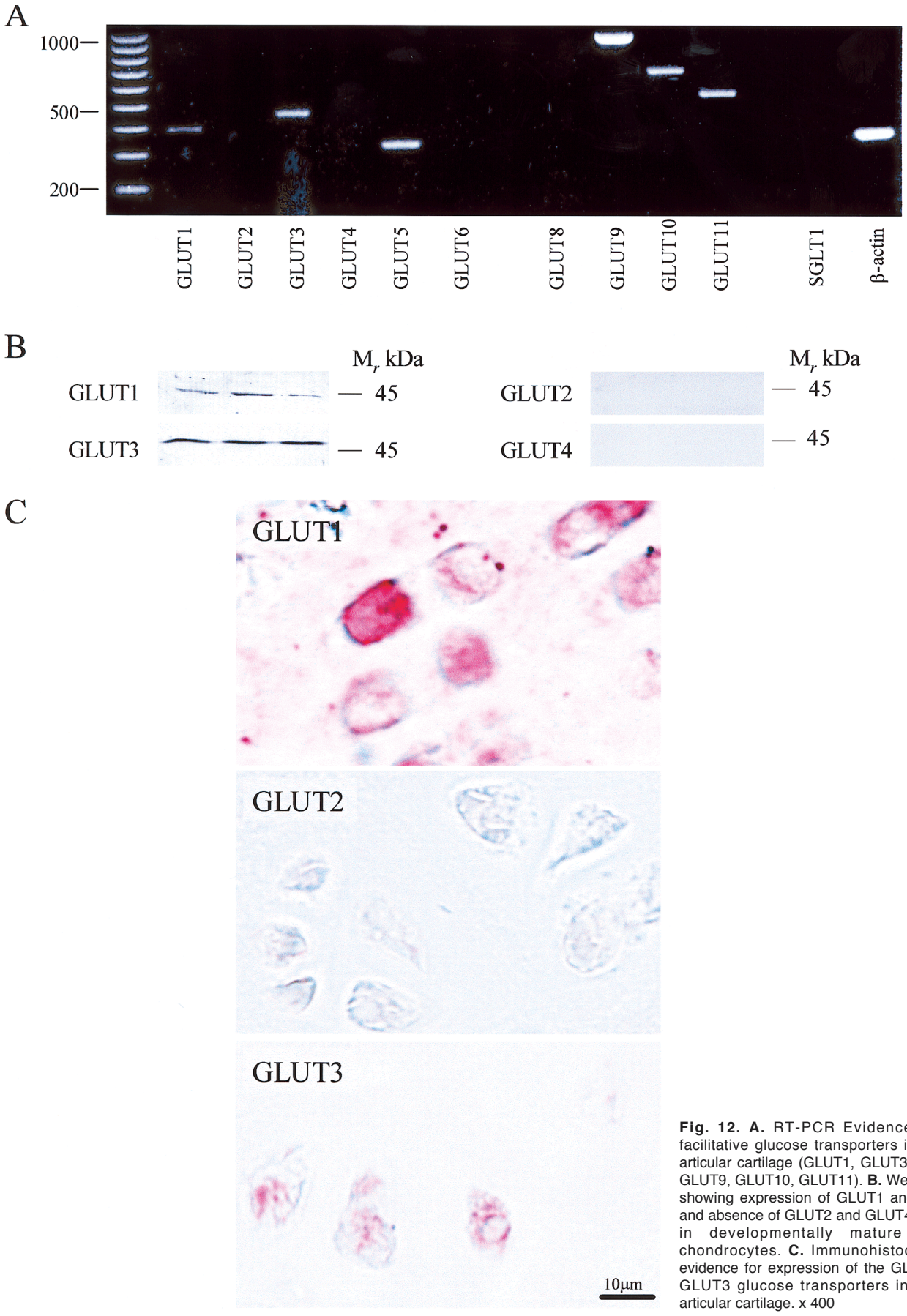

Fig. 12. A. RT-PCR Evidence for six facilitative glucose transporters in human articular cartilage (GLUT1, GLUT3, GLUT5, GLUT9, GLUT10, GLUT11). **B.** Western blot showing expression of GLUT1 and GLUT3 and absence of GLUT2 and GLUT4 proteins in developmentally mature human chondrocytes. **C.** Immunohistochemical evidence for expression of the GLUT1 and GLUT3 glucose transporters in porcine articular cartilage. x 400

was 100% identical to sequences in GenBank corresponding to the Homo sapiens facilitated glucose transporter, member 3, GLUT3 (SLC2A3). The partial sequence was deposited in GenBank under accession number AY034634. The GLUT9 sequence was 100% identical to recently deposited sequences in GenBank corresponding to the Homo sapiens facilitated glucose transporter, member 9, GLUT9 (SLC2A9). The partial sequence was deposited in GenBank under accession number AF421859 (Mobasheri et al., 2002a). We have

Table 2. Oligonucleotide primer pairs used to detect the presence of mRNAs encoding members of the GLUT family (SLC2A) of facilitative glucose transporters in human articular cartilage by PCR (see Fig. 12). The cDNA for GLUT7 (SLC2A7) has not yet been cloned. Expression of GLUT7 and GLUT12 was not investigated. The primer list also includes the sodium dependent glucose carrier SGLT1 (SLC5A1), the Na^+ dependent glucose transporter expressed in the intestine and kidney. Human ß- actin (HUMACTA1) primers were included in all PCR experiments as an internal control. The homology of the sequenced PCR products to known sequences in the database was investigated using BLASTN 2.2.1 (http://www.ncbi.nlm.nih.gov/BLAST/). The partial cDNA sequences obtained from human chondrocytes were submitted to GenBank.

GENE	OLIGONUCLEOTIDE PRIMERS 5' – 3' direction	Tm °C	PRODUCT SIZE (bp)	HOMOLOGY TO KNOWN SEQUENCES
GLUT1 (SLC2A1)	(F) TCC ACG AGC ATC TTC GAG A (R) ATA CTG GAA GCA CAT GCC C	58 58	392	100% identical to nucleotides 1052 to 1450 of the Homo sapiens solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1), mRNA (Accession number: XM_002033.4) Partial sequence submitted to GenBank as AY034633
GLUT2 (SLC2A2)	(F) CAC TGA TGC TGC ATG TGG C (R) ATG TGA ACA GGG TAA AGG CC	60 60	521	Not detected in mature human cartilage
GLUT3 (SLC2A3)	(F) TTC AAG AGC CCA TCT ATG CC (R) GGT CTC AGG GAC TTT GAA GA	60	457	100% identical to nucleotides 1180 to 1599 of the *Homo* sapiens solute carrier family 2 (facilitated glucose transporter), member 3 (SLC2A3), mRNA (Accession number: XM_049663.1) Partial sequence submitted to GenBank as AY034634
GLUT4 (SLC2A4)	(F) GGC ATG TGT GGC TGT GCC ATC (R) GGG TTT CAC CTC CTG CTC TAA	61 53	413	Not detected in mature human cartilage
GLUT5 (SLC2A5)	(F) CCC TTG ACG TTG CTG TGGT (R) ACA GTG ATG AAG AGC TGG GG	60 62	322	98% similarity to nucleotides 247 to 566 of the Homo sapiens solute carrier family 2 (facilitated glucose/fructose transporter), member 5 (SLC2A5), mRNA (Accession number: XM_045269.2) Partial sequence submitted to GenBank as AF479408
GLUT6 (SLC2A6)	(F) GGG TGT TCC TGG CCA CC (R) GCC TGG GGG AGC AAT CTC	58 60	350	Not detected in mature human cartilage
GLUT7 (SLC2A7)			--	Not studied
GLUT8 (SLC2A8)	(F) CAT GCT GCT TCT CAT GTG C (R) CAT GAG CCA GTT GGT GAG G	58 60	681	Not detected in mature human cartilage
GLUT9 (SLC2A9)	(F) CATCAAGGCCTTTTACAATGAGT (R) AAGCCACCAATGAGGAGG	52 56	900	99% similarity to nucleotides 306 to 902 of the *Homo sapiens* solute carrier family 2 (facilitated glucose transporter), member 9 (SLC2A9), mRNA (Accession number: NM_020041.1) Partial sequence submitted to GenBank as AF421859
GLUT10 (SLC2A10)	(F) GGT CTT TGT CAG TGC CTT CT (R) GAG ATG TGC AAG TCA ATG GG	60 60	633	100% identical to nucleotides 1500 to 2131 of the Homo sapiens solute carrier family 2 (facilitated glucose transporter), member 10 (SLC2A10), mRNA (Accession number: XM_029983.1) Partial sequence submitted to GenBank as AF479407
GLUT11 (SLC2A11)	(F) GCG CTG CCG GCA TTG GTG GG (R) CAC CTA GGA GCT CCC TGA G	70 62	501	100% identical to nucleotides 151 to 649 of the Homo sapiens solute carrier family 2 (facilitated glucose transporter), member 11 (SLC2A11), mRNA (Accession number: XM_037696.1) Partial sequence submitted to GenBank as AF479409
GLUT12 (SLC2A12)			---	Not studied
SGLT1 (SLC5A1)	(F) TCT TCG ATT ACA TCC AGT CCA (R) TCT CCT CTT CCT CAG TCA TC	50 60	521	Not detected in mature human cartilage
ß-actin (HUMACTA1)	(F) TTC AAC TCC ATC ATG AAG TGT GAC GTG (R) CTA AGT CAT AGT CCG CCT AGA AGC ATT	61 57	309	Housekeeping gene; used as internal PCR control

since obtained molecular evidence for the expression of an additional three GLUTs in human chondrocytes: GLUT5, GLUT10 and GLUT11. We have completed cloning and sequencing of the partial cDNAs corresponding to the GLUTs shown in Fig. 12 and the partial sequences have submitted to GenBank (see Table 1, Fig. 12A). We have not been able to detect expression of GLUT2, GLUT4 or the sodium-dependent glucose transporter SGLT1 in any human cartilage samples or cDNA libraries examined to date. Expression of GLUT1 and GLUT3 protein and the absence of GLUT2 and GLUT4 in human chondrocytes have been confirmed by Western blotting (Fig. 12B). Studies employing fluorescence activated cell sorting (FACS analysis) demonstrated that GLUT1 and GLUT3 are expressed by more than 90 % of monolayer human chondrocytes examined (Fig. 13). We have also used indirect immunofluorescence to confirm that GLUT1 and GLUT3 proteins are found in the plasma membrane of human chondrocytes (Neama et al., 2001; Mobasheri et al., 2001, 2002a). We have not yet determined whether GLUT5 protein is present in chondrocytes. As far as we are aware, human-specific antibodies to the recently identified GLUT members (GLUT6, GLUT7, GLUT8, GLUT9, GLUT10, GLUT11 and GLUT12) have not yet been developed (Shikhman et al., 2001). With suitable antibodies in hand, future studies will determine the expression, distribution and regulation of GLUT5,

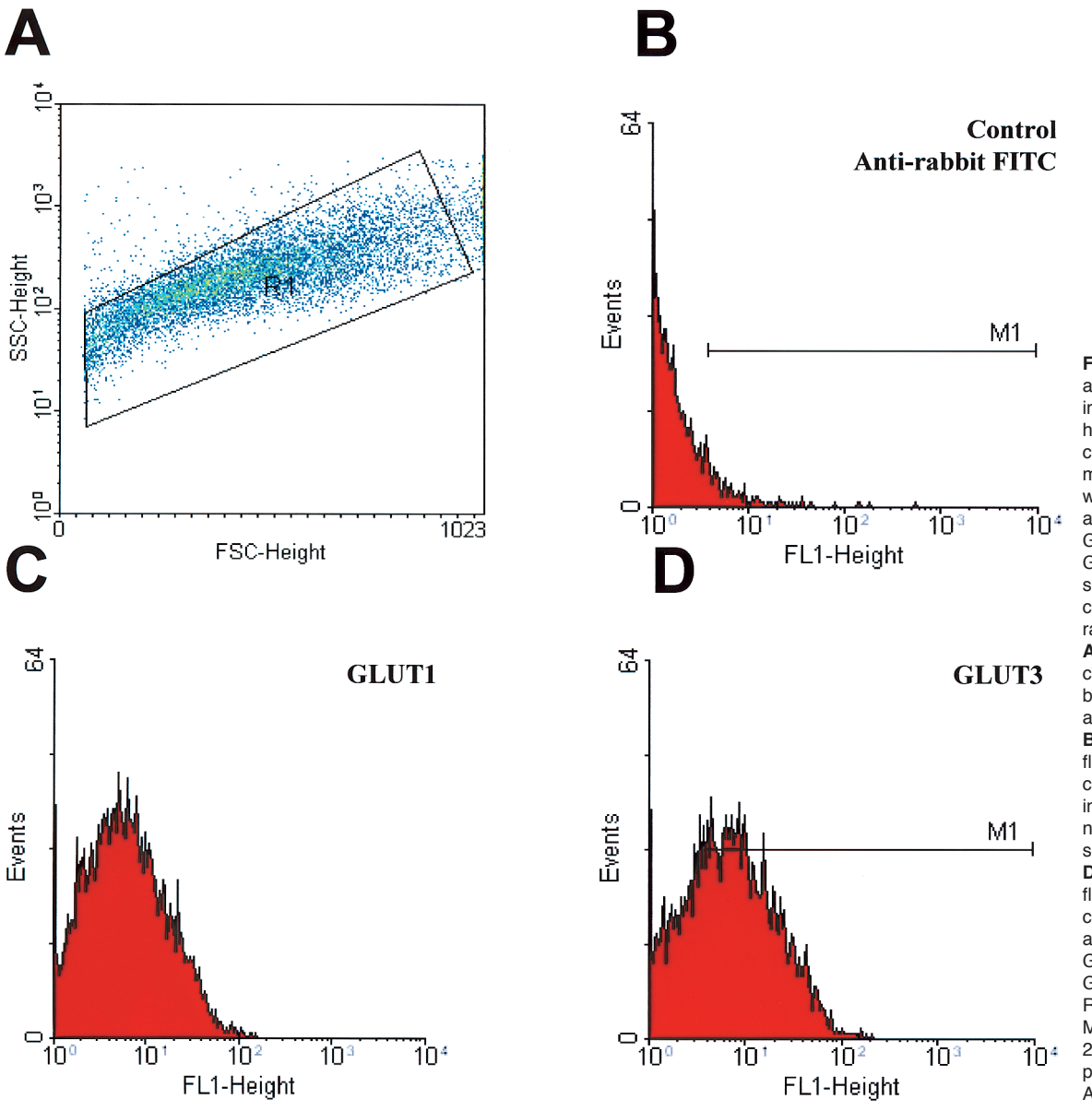

Fig. 13. FACS analysis of immortalized human chondrocytes in monolayer probed with polyclonal antibodies to GLUT1 and GLUT3 and secondary FITC-conjugated anti-rabbit IgG. **A:** Scatter plot of chondrocytes based on cell size and granularity. **B:** Background fluorescence from control cells incubated with non-immune serum. **C and D:** Specific fluorescence from cells probed with antibodies to GLUT1 and GLUT3. Reproduced from Mobasheri et al., 2002a with kind permission of Academic Press.

GLUT9, GLUT10 and GLUT11 in chondrocytes from normal and diseased cartilage.

Cytokine regulation of chondrocyte GLUTs

Hernvann and co-workers (1996) were the first to study glucose transport in chondrocytes and observed accelerated glucose uptake in chondrocytes and synoviocytes in response to IL-1ß stimulation. Furthermore, they showed that the uptake is completely inhibited by cortisol (Hernvann et al., 1996). This inhibition of glucose uptake by cortisol may partially account for some of its anti-inflammatory effects in rheumatoid patients. Recent data from the Lotz laboratory supports these earlier observations and provides additional evidence for up to four glucose transporters in human cartilage (Shikhman et al., 2001b). From their experiments in human chondrocytes they suggest that GLUT3 and GLUT8 are constitutively expressed, whereas GLUT1 and GLUT9 are more responsive to cytokine stimulation and are therefore "inducible". They also claim that IL-1ß enhances the expression and plasma membrane incorporation of the highly glycosylated form of GLUT1 (the 55 kDa form normally found in erythrocytes) in chondrocytes. In other studies the highly glycosylated form of GLUT1 has been shown to exhibit an increased protein stability, and an increased rate of protein incorporation into the plasma membrane than its non-glycosylated analog (the 45 kDa form of GLUT1; Asano et al., 1991). Post-translational glycosylation appears to be necessary for full glucose transport activity, intracellular targeting and GLUT1 protein stability (Asano et al., 1991, 1993). However, the physiological role of additional levels of GLUT1 glycosylation has not been fully determined. Despite these important observations, Shikhman and colleagues (2001b) have not examined the expression of all the known GLUTs; expression of GLUT5, GLUT6, GLUT10 and GLUT11 was not investigated at the mRNA or protein levels. Furthermore, they suggest that GLUT1 is inducible by pro-inflammatory cytokine treatment (IL-1ß), but is not constitutively expressed in cartilage. Our studies suggest that GLUT1 mRNA and protein is constitutively present in human and equine chondrocytes in addition to mRNA and protein for GLUT3 (Mobasheri et al., 2002a) and mRNA for GLUT5, GLUT 10 and GLUT11 (Mobasheri et al., 2002c). There are three possible explanations for the apparent differences in the data obtained by the two laboratories. First, there may have been differences in the cDNA libraries used for PCR. Second, the PCR primers used in these two studies were different. Third, different commercial antibodies were used in both of these studies. Despite the apparent differences, there are important similarities in our findings and the fact remains that articular chondrocytes may express up to four or six facilitative glucose transporters. We have evidence that GLUT1 and GLUT3 are constitutively expressed in human (Neama et al., 2001; Mobasheri et al., 2001, 2002a) ovine and equine chondrocytes (Mobasheri et al., unpublished observations). Despite these differences, it appears that multiple GLUT isoforms are expressed in chondrocytes, suggesting that glucose uptake is specialized in these cells. Above all, the expression of GLUT1, GLUT3 and GLUT9 will have important functional consequences for cartilage. The extent to which these proteins may be involved in the facilitated uptake of glucose, as well as fructose and glucose-derived compounds such as glucosamine sulfate and the oxidized form of vitamin C, dehydroascorbate that is essential for ECM synthesis, is of great interest and requires future clarification.

Functional significance of multiple GLUT/ SLC2A members in cartilage

The presence of GLUT1 in cartilage was expected as this glucose transporter is ubiquitously expressed in most adult and foetal tissues as a "housekeeping" isoform (Mueckler, 1994). Expression of GLUT3 in cartilage was surprising as the tissue distribution of this transporter is limited and it is normally found primarily in neurons, but also in the testes and spermatozoa, placenta and human platelets (Haber et al., 1993; Maher et al., 1994; Vannucci et al., 1997). GLUT3 expression in neurons has been suggested to be related to neuronal maturation and synaptogenesis, as well as to chronic changes in neuronal activity both in vivo and in vitro (Maher and Simpson, 1994; Vannucci, 1994; Vannucci et al., 1994a,b).

Early kinetic studies by Gould et al. (1991) suggest that the GLUT3 transporter is a high affinity transporter (exhibiting low K_m of approx. 1 mM) and thus it may have evolved to operate more efficiently at lower substrate concentrations, such as those encountered in the brain. Glucose transport in brain can be viewed as a multistep process involving different glucose transporters that exhibit distinct kinetics (Carruthers, 1990; Vannucci et al., 1997). Thus, glucose transport across the blood brain barrier (BBB) involves movement of glucose from the lumen of the microvessels into the endothelial cells, and then across the abluminal membrane into the interstitium where it is made available as an energy substrate for neurons and glia. The glucose gradient out of the endothelial cells and into the interstitium is significant (estimated glucose concentrations in the interstitium are in the range of 1-2 mM (Dienel et al., 1991; Greutter et al., 1992). Studies comparing GLUT1 and GLUT3 kinetics in the brain suggest that the affinity of these transporters for glucose is high (close to the prevailing interstitial glucose concentration (i.e. 1.7 mM, GLUT1 and 2.8 mM GLUT3). In real terms, what differentiates the two transporter isoforms is the "rate" at which they transport glucose or more precisely their catalytic center activity values: 123 s-1, GLUT1 and 853 s-1, GLUT3. Thus GLUT3 transports glucose seven times faster than GLUT1 (Maher et al., 1996). The most obvious

interpretation of these differences is that a neuron expressing an equal number of GLUT3 transporters has a greater capacity to transport glucose than a glial cell with the same number of GLUT1 transporters.

Like neurons and glia, chondrocytes also exist in a unique interstitium where glucose and oxygen concentrations may fall to very low levels. However, unlike the central nervous system, mature cartilage is avascular. Therefore, in cartilage, GLUT3 may operate more efficiently (i.e. faster) than other GLUTs at low substrate concentrations and this may reflect a possible metabolic adaptation where glucose is a major metabolic fuel in short supply under physiological conditions. The sodium-dependent glucose transporter SGLT1, which is responsible for glucose absorption in enterocytes (Hediger et al., 1989; Wright et al., 1992) in the intestine and proximal tubule cells in the kidney, was not detected in human chondrocytes (Mobasheri et al., 2002a). We found this surprising as we had originally hypothesized that the existence of a steep inward Na^+ gradient into chondrocytes in articular cartilage could be exploited by these cells to co-transport essential nutrients. Although SGLT1 is not present in chondrocytes, SGLT2 and SGLT3 isoforms may be expressed in chondrocytes and future studies will undoubtedly confirm whether SGLT2 and SGLT3 are expressed in cartilage. The high-capacity, low affinity GLUT2 was also undetectable in mature human cartilage together with GLUT6, GLUT4 and GLUT8. This observation from our laboratory is in agreement with the findings of Shikhman et al., (2001b) where GLUT2 and GLUT4 mRNA was not detected in chondrocytes. Since GLUT9 was recently cloned, no information is currently available about its kinetic properties and cellular expression patterns (Phay et al., 2000). The same is true of the other newly identified GLUTs. There is however some kinetic data for GLUT10. Dawson et al. (2001) suggest that this GLUT isoform exhibits a very low K_m of approx. 0.3 mM and is therefore a very "fast" transporter. This kinetic property of GLUT10 would be very suited to the unusual

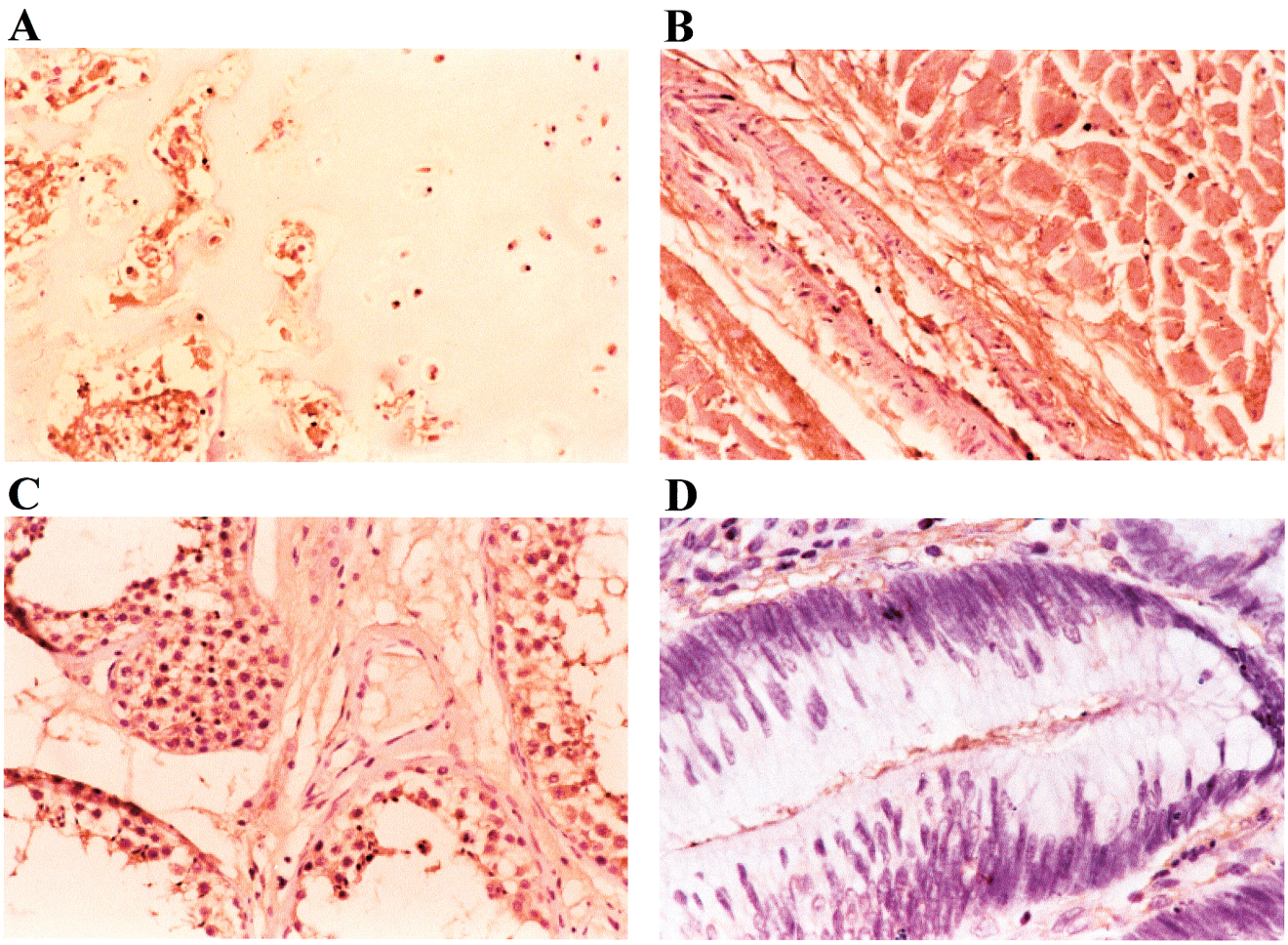

Fig. 14. Immunoperoxidase demonstration of monocarboxylate transporter MCT-1 expression in porcine cartilage **(A),** human heart **(B)**, human testis **(C)** and human large intestine **(D)**. The brown staining (DAB) corresponds to areas of high MCT-1 protein expression. Cell nuclei were counter-stained with hematoxylin. x 200

circumstances of the chondrocyte, where glucose is a relatively scarce resource in fully developed, avascular cartilage.

GLUT1 and hypoxia

Glucose transport is acutely stimulated by hypoxic conditions where the response to hypoxia is mediated by enhanced expression and function of class I facilitative glucose transporters (GLUT1, GLUT3 and GLUT4; Zhang et al., 1999). Hypoxia and oncogenic transformation of cultured cells induce GLUT1 mRNA by upregulating hypoxia-inducible factor-1 (Behrooz and Ismail-Beigi, 1997; Chen et al., 2001). Acute (cerebral hypoxia-ischemia) and chronic (hypobaric hypoxia) situations *in vivo* increase GLUT1 levels in the blood-brain barrier (Harik et al., 1994; Vannucci et al., 1996, 1998), through up-regulation of Hif-1. Hif-1α mediated increases in BBB GLUT1 have recently been shown to contribute to the phenomenon of hypoxic preconditioning in the immature rat (Jones and Bergeron, 2001). Oxygen tensions in articular cartilage are generally very low (Otte, 1991; Lee and Urban, 1997). As for all cells, in the absence of oxidative phosphorylation, chondrocytes must rely on glycolysis for ATP production (Otte, 1991). Consequently, low oxygen tensions and hypoxia may influence glucose uptake and metabolism by Hif-1 stimulation expression of GLUT1, and perhaps also GLUT3, in articular chondrocytes. This situation will particularly apply to those cells situated a long distance from the synovial microcirculation in the deep zone (Otte, 1991).

Monocarboxylate transporters in chondrocytes

Monocarboxylic acids play a major role in the metabolism of all living cells. Lactic acid, the end product of glucose metabolism in the glycolytic pathway, is a monocarboxylate and together with pyruvate plays an important role in cellular metabolism. Erythrocytes, skeletal muscle cells and most tumour cells depend on the glycolytic pathway for the production of ATP under normal physiological conditions (Halestrap and Price, 1999). Chondrocytes utilize the glycolytic pathway and produce lactate, which is subsequently released into the surrounding matrix (Pickart and Thaler, 1980; Munteanu et al., 2000). Although lactate is understood to be a by-product of glycolysis in chondrocytes, there is no published information on how it is transported across the chondrocyte plasma membrane.

Monocarboxylate transporters are a family of integral membrane proteins that mediate proton-coupled co-transport of monocarboxylic acids, i.e. lactate, pyruvate, and the ketone bodies b-hydroxybutyrate and acetoacetate, across the cell membrane (Halestrap and Price, 1999). MCT1 is important for lactate efflux in muscle cells (von Grumbckow et al., 1999; Pilegaard et al., 1999) and MCT4, another member of the monocarboxylate transporter family is specifically adapted to the export of lactate in highly glycolytic cells such as active myocytes (Dimmer et al., 2000). Preliminary work using polyclonal antibodies to MCT1 has revealed that this monocarboxylate transporter is expressed in chondrocytes in addition to cardiac muscle, the testes and the large intestine (Mobasheri, unpublished observations; Fig. 14). It is clear that chondrocytes require a transport mechanism for lactate efflux and we propose that MCT1 is involved in this specific function which would dispose of lactate generated by anaerobic metabolism and regulate intracellular pH in these cells. There is still much work to be done to characterize lactate transport in these cells and identify other MCT isoforms that may be involved.

Altered glucose handling in connective tissue disorders: A hypothesis

Extremes of blood glucose and disturbances in oxygen supply cause complications for a variety of glucose sensitive tissues and organs. Since cartilage is highly dependent on glucose as a metabolic fuel and structural component, we hypothesize that extremes of blood glucose (and hence tissue/synovial fluid glucose) can cause complications for bones and joints. We also propose that endocrine disorders (whether genetic or environmental) that affect the levels of circulating hormones (i.e. IGFs, GH and leptin) can influence glucose metabolism and the bioenergetic state of chondrocytes and other cell types in connective tissues. Low blood glucose levels and hypoxic conditions would mean lower synovial and subchondral glucose for highly metabolic articulating cartilage. We also propose that high synovial and subchondral glucose levels are equally problematic resulting in glycation of matrix proteins leading to joint pathology and in such cases the excess glucose may act as a competitive inhibitor of vitamin C by GLUT1 thus reducing the intracellular levels of vitamin C in chondrocytes. The competitive inhibition of vitamin C uptake (or rather dehydroascorbic acid) by excess glucose may, in the long term, exert deleterious effects on collagen synthesis and cross-linking in cartilage. This hypothesis is consistent with recent evidence for the involvement of glucose in abnormalities of interstitial connective tissues involving skeleton, joints, skin, and peri-articular tissues where advanced glycation end products are formed as a result of non-enzymatic reaction of glucose with proteins, resulting in connective tissue disorders (Rosenbloom and Silverstein, 1996).

Glucose-derived nutraceuticals

Glucosamine sulfate is an amino sugar that is believed to play a role in cartilage formation and repair. It is a naturally occurring substance in the body essential for the normal growth and repair of connective tissue, joints and articular cartilage. It has been proposed that

glucosamine sulfate helps rebuild damaged joints, tendons, cartilage and soft tissue and supplements containing this compound have been used to treat osteoarthritis in horses and dogs for several years and in Europe, glucosamine has been prescribed to treat osteoarthritis since the early 1980's. Glucosamine exists in several forms, usually either as glucosamine sulfate or glucosamine hydrochloride. Both forms have been reported to work well, although most experimental studies have been done with the sulfate form. Laboratory tests show it is 98% absorbable and because the molecules are small and simple, they are actually able to penetrate the cartilage matrix, where they are absorbed by chondrocytes and incorporated into matrix proteins. More than 90% of orally administered glucosamine sulfate is absorbed and deposited in articular cartilage. The scheme shown in Fig. 7 shows the fate of glucose and glucosamine in the glycosaminoglycan synthesis pathway. Pharmacological effects of glucosamine sulfate in chondrocytes (*in vitro*) include increased proteoglycan synthesis (Bassleer et al., 1992), increased aggrecan core protein mRNA expression, decreased cell viability and decreased GAG production (Anderson et al., 1999), inhibition of nitric oxide production, inhibition of MMPs and aggrecanases. Glucosamine sulfate also has an attractive safety profile and in animal models it has been shown to exert weak anti-inflammatory responses (Setnikar, 2001; Setnikar and Rovati, 2001). In terms of OA symptom modification, clinical trials in human OA have been poorly designed, are slightly biased and show weak to moderate pain relieving effects (McAlindon et al., 2000). There are no clinical trials in veterinary species. Glucosamine sulfate has been reported to function as a mild to moderate modifier of cartilage structure and function in OA of the knee in humans (Reginster et al., 2000, 2001). There is also evidence for beneficial effects of combination products containing glucosamine sulfate, chondroitin sulfate, manganese and ascorbate (Cosequin®: CS-G-M) in a canine model of OA where decreased cartilage lesions have been observed in the combination treated dogs (Johnson et al., 2001). CS-G-M is able to influence the expression of chondroitin sulfate epitopes in synovial fluid with similar effects in the contralateral joint.

Other related polysulfate nutraceuticals

Pentosan polysulfates (sodium pentosan polysulfate, NaPPS; calcium pentosan polysulfate, CaPPS) have also been proposed as structure-modifying osteoarthritis (OA) drugs (SMOADS) (Munteanu et al., 2000). Both NaPPS and CaPPS have been shown to exhibit pharmacological activities such as the ability to support chondrocyte anabolic activities and particularly the propensity to attenuate catabolic events in OA joints (for a review see Ghosh, 1999). In addition to suppressing MMP expression and activity, other beneficial effects of NaPPS and CaPPS are anti-inflammatory: stimulation of tissue plasminogen activator, superoxide dismutase and lipases with the net thrombolytic and lipolytic effect of improving blood flow to the joint capsule and the subchondral capillaries. Ultimately NaPPS and CaPPS may serve to improve cartilage and bone cell nutrition and hence they have been classified as SMOADs. Polysulfate nutraceuticals are likely to feature in combination SMOAD products developed in the future.

Implications and consequences

Glucose transporters represent the rate-limiting step for glucose uptake across the cell membrane. In chondrocytes they are responsible for maintaining a steady supply of glucose and related substances in order to preserve cartilage integrity. In this review article we have considered the role of glucose transport systems expressed by chondrocytes in the context of their nutritional, metabolic and developmental role in cartilage. We have also discussed the role of endocrine disease in the pathogenesis of osteoarticular disorders and presented a novel hypothesis that implicates facilitative glucose transporters in cartilage development and the onset of degenerative joint disorders.

The pathogenesis of OA is traditionally thought to originate in the synovial joint and is not generally thought to be influenced by systemic, endocrine and metabolic disturbances. This dogmatic view is inconsistent with recently published data that demonstrate distorted GH/IGF-I axis in the symptomatic OA patient (Denko and Malemud, 1999). In light of this novel information and the discovery of leptin receptors on chondrocytes in developing and mature cartilage (Figenschau et al., 2001; Kume et al., 2002), the role of circulating GH, leptin, IGF-I and other growth factors that regulate the chondrocyte's biosynthetic activities and glycolytic metabolism in the pathogenesis and progression of OA requires further clarification. In addition, the contribution of nutrient transporters such as facilitative glucose transporters needs to be considered in the context of the physico-chemical environment of chondrocytes and the paucity of nutrients therein. There is a connection between elevated GH and decreased IGF-I levels (Denko and Malemud, 1999). In addition, there is a link between excess circulating IGF-I, GH and major joint pathology in acromegaly (Lioté and Orcel, 2000; Stavrou and Kleinberg, 2001). The weakened IGF-I response in OA coupled with elevated cartilage ECM degradation (mediated by metalloproteinases) and depressed compensatory biosynthesis (induced and perpetuated by the presence of pro-inflammatory catabolic cytokines such as IL-1ß and TNFα may, in fact, act synergistically to suppress normal cartilage repair responses thus resulting in progressive destructive lesions of articular cartilage and subchondral bone.

Glucose serves as a major energy source (Otte, 1991; Lee and Urban, 1997) and as a principal precursor for the synthesis of glycosaminoglycans (Kim and Conrad, 1976; Sweeney et al., 1993). For this reason the increased glucose uptake observed in chondrocytes in

response to IFG-I/insulin and cytokine stimulation may be due to the mutual dependency of both anabolic and catabolic pathways on regulated glucose transport (see Fig. 15). Chondrocytes require the energy derived from glucose whether they are synthesizing or degrading ECM macromolecules. However, despite the apparent similarities in glucose consumption in response to anabolic and catabolic stimulators the subcellular signalling pathways and the long-term consequences for matrix stability will be significantly different. The accelerated glucose transport observed following catabolic IL-1ß/TNF-α stimulation will result in accumulation of intracellular glucose but in this case the accumulated substrate is likely to be channelled mainly into metabolic pools in order to generate the ATP required by chondrocytes to degrade the ECM. Chondrocytes stimulated by anabolic factors like IGF-I or insulin may divert accumulated glucose into metabolic and structural pools. Studies in synovial cells, chondrocytes and fibroblasts have demonstrated that chemokines such as connective tissue activating peptide III increase the rate of glucose transport in these cells and upregulate GLUT1 mRNA and protein (Ku Tai et al., 1992). Thus it would appear that stimulated glucose transport and its subsequent utilization represent an important component of the chondrocyte response to both anabolic and catabolic mediators (Bird et al., 1990; Ku Tai et al., 1992). Stimulated glucose transport and upregulation of GLUT proteins is likely to be an early and sustained event in inflammatory and wound healing processes in connective tissues. However, the subcellular signalling pathways activated by these diverse stimuli are poorly understood. The challenge is to identify the molecular participants of these signalling cascades and to determine whether there are any signalling alterations in degenerative joint disease. We also need to determine whether glucose transport and metabolism in chondrocytes is regulated by circulating and local endocrine factors such as IGFs, IGFBPs and leptin. This understanding may reveal novel therapeutic targets (either the inducible glucose transporters themselves or their regulators), which may enable clinicians to preferentially thwart the catabolic events that occur in OA and OCD.

Conclusions

Given the central role of glucose in normal chondrocyte physiology and metabolism *in situ*, its regular provision via GLUTs is likely to have a profound influence on the metabolic activity and survival of chondrocytes in articular cartilage. Glucose-derived sugars, other hexoses, pentoses and sulfated sugars may be transported by a variety of GLUT isoforms that

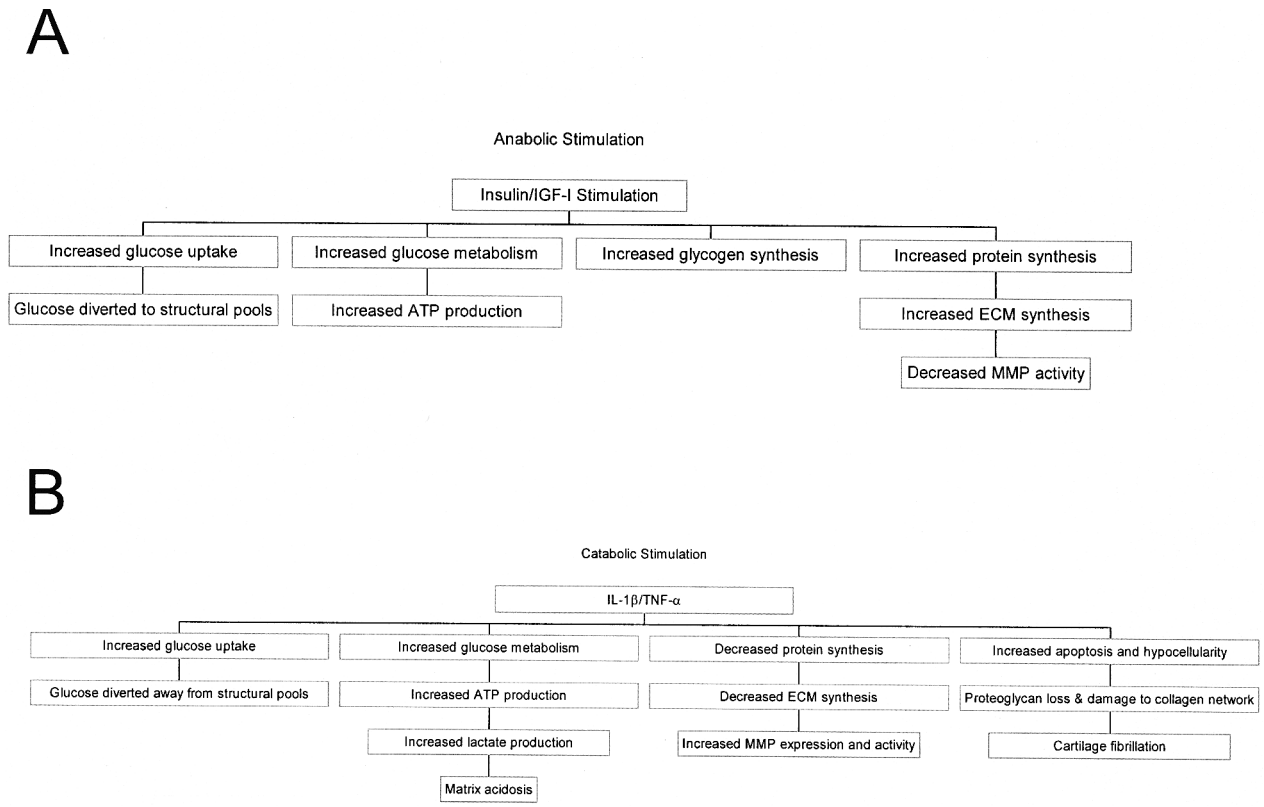

Fig. 15. Proposed pathways of glucose transport and metabolism in chondrocytes stimulated with anabolic **(A)** and catabolic **(B)** mediators of cartilage turnover.

exhibit diverse substrate specificities. Future studies will need to determine whether there are changes to the pattern of GLUT isoform expression in OA and OCD compared to normal cartilage. Also, GLUTs may become suitable therapeutic targets for modulating the phenotypic characteristics of cells in cartilage and in artificial cartilage constructs with the aim of improving the biomechanical performance and durability of the engineered tissue. Nutritional supplements, vitamins, nutraceuticals and pharmacological modulation of nutrient transporters may be of benefit in the treatment of OA (Fillmore et al., 1999; Haugen et al., 1999) and it is feasible that improved nutritional support of chondrocytes will prevent and alleviate OA (Willhelmi, 1993a,b). Any data leading to identification of effects of nutrition on chondrocyte physiology will have a major impact on the prevention and treatment of a range of rheumatological conditions.

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References

Adams C.S. and Horton W.E. (1998). Chondrocyte apoptosis increases with age in the articular cartilage of adult animals. Anat. Rec. 250, 418-425.

Agus D.B., Gambhir S.S., Pardridge W.M., Spielholz C., Baselga J., Vera J.C. and Golde D.W. (1997). Vitamin C crosses the blood-brain barrier in the oxidized form through the glucose transporters. J. Clin. Invest. 100, 2842-2848.

Agus D.B., Vera J.C. and Golde D.W. (1999). Stromal cell oxidation: a mechanism by which tumors obtain vitamin C. Cancer Res. 59, 4555-4558.

Anderson C.C., Cook J.L., Kreeger J.M., Tomlinson J.L. and Wagner-Mann C.C. (1999). *In vitro* effects of glucosamine and acetylsalicylate on canine chondrocytes in three-dimensional culture. Am. J. Vet. Res. 60, 1546-1551.

Asano T., Katagiri H., Takata K., Lin J.L., Ishihara H., Inukai K., Tsukuda K., Kikuchi M., Hirano H. and Yazaki Y. (1991). The role of N-glycosylation of GLUT1 for glucose transport activity. J. Biol. Chem. 266, 24632-24636.

Asano T., Takata K., Katagiri H., Ishihara H., Inukai K., Anai M., Hirano H., Yazaki Y. and Oka Y. (1993). The role of N-glycosylation in the targeting and stability of GLUT1 glucose transporter. FEBS Lett. 324, 258-261.

Avruch J. (1998). Insulin signal transduction through protein kinase cascades. Mol. Cell. Biochem. 182, 31-48.

Bassleer C., Henrotin Y. and Franchimont P. (1992). *In vitro* evaluation of drugs proposed as chondroprotective agents. Int. J. Tissue React. 14, 231-241.

Behrooz A. and Ismail-Beigi F. (1997). Dual control of GLUT1 glucose transporter gene expression by hypoxia and by inhibition of oxidative phosphorylation. J. Biol. Chem. 272, 5555-5562.

Beitz E. and Schultz J.E. (1999). The mammalian aquaporin water channel family: A promising new drug target. Curr. Med. Chem. 6, 457-467.

Bell G.I., Kayano T., Buse J.B., Burant C.F., Takeda J., Lin D., Fukumoto H. and Seino S. (1990). Molecular biology of mammalian glucose transporters. Diabetes Care. 13, 198-208.

Bellows C.G., Aubin J.E., Heersche J.N. and Antosz M.E. (1986). Mineralized bone nodules formed in vitro from enzymatically released rat calvaria cell populations. Calcif. Tissue Int. 38, 143-154.

Benya P.D. and Shaffer J.D. (1982). Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell. 30, 215-224.

Bernick S. and Cailliet R. (1982). Vertebral end-plate changes with aging of human vertebrae. Spine 7, 97-102.

Bhaumick B. and Bala R.M. (1991). Differential effects of insulin-like growth factors I and II on growth, differentiation and glucoregulation in differentiating chondrocyte cells in culture. Acta Endocrinol. 125, 201-211.

Bird T.A., Davies A., Baldwin S.A. and Saklatvala J. (1990). Interleukin 1 stimulates hexose transport in fibroblasts by increasing the expression of glucose transporters. J. Biol. Chem. 265, 13578-13583.

Blanco F.J., Ochs R.L., Schwarz H. and Lotz M. (1995). Chondrocyte apoptosis induced by nitric oxide. Am. J. Pathol. 146, 75-85.

Blanco F.J., Guitian R., Vazquesmartul E., deToro F.J. and Glado F. (1998). Osteoarthritis chondrocytes die by apoptosis. Arthritis Rheum. 41, 284-289.

Boullion R. (1990). Diabetic bone disease. Calcif. Tissue Int. 49, 155-160.

Brighton C.T., Ray R.D. and Soble L.W. (1969). In vitro epiphysial plate growth in various oxygen tensions. J. Bone Joint Surg. 1A, 1383-1396.

Brighton C.T., Sugioka Y. and Hunt R.M. (1973). Cytoplasmic structures of epiphyseal plate chondrocytes. Quantitative evaluation using electron micrographs of rat costochondral junctions with special reference to the fate of hypertrophic cells. J. Bone Joint Surg. Am. 55, 771-784.

Brighton C.T., Lackman R.D. and Cuckler J.M. (1983). Absence of the glycerol phosphate shuttle in the various zones of the growth plate. J. Bone Joint Surg. 65A, 663-666.

Buckwalter J.A., Martin J. and Mankin H.J. (2000). Synovial joint degeneration and the syndrome of osteoarthritis. Instr. Course Lect. 49, 481-489.

Buckwalter J.A. and Mankin H.J. (1998a). Articular cartilage repair and transplantation. Arthritis Rheum. 41, 1331-1342.

Buckwalter J.A. and Mankin H.J. (1998b). Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr. Course Lect. 47, 477-486.

Buckwalter J.A. and Mankin H.J. (1998c). Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr. Course Lect. 47, 487-504.

Burant C.F. and Davidson N.O. (1994). GLUT3 glucose transporter

isoform in rat testis: localization, effect of diabetes mellitus, and comparison to human testis. Am. J. Physiol. 267, R1488-R1495.

Burkus J.K., Ganey T.M. and Ogden J.A. (1993). Development of the cartilage canals and the secondary center of ossification in the distal chondroepiphysis of the prenatal human femur. Yale J. Biol. Med. 66, 193-202.

Carayannopoulos M.O., Chi M.M.Y., Cui Y., Pingsterhaus J.M., McKnight R.A., Mueckler M., Devaskar S.U. and Moley K.H. (2000). GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst. Proc. Natl. Acad. Sci. USA 13, 7313-7318.

Carruthers A. (1990). Facilitated diffusion of glucose. Physiol. Rev. 70, 1135-1176.

Catterall W.A. (1999). Molecular properties of brain sodium channels: an important target for anticonvulsant drugs. Adv. Neurol. 79, 441-456.

Chappard D., Alexandre C. and Riffat G. (1986). Uncalcified cartilage resorption in human fetal cartilage canals. Tissue Cell 18, 701-707.

Chen C., Pore N., Behrooz A., Ismail-Beigi F. and Maity A. (2001). Regulation of GLUT1 mRNA by hypoxia-inducible factor-1. Interactions between H-ras and hypoxia. J. Biol. Chem. 276, 9519-9525.

Clark J.M. (1990). The structure of vascular channels in the subchondral plate. J. Anat. 171, 105-115.

Cloherty E.K., Sultzman L.A., Zottola R.J. and Carruthers A. (1995). Net sugar transport is a multistep process. Evidence for cytosolic sugar binding sites in erythrocytes. Biochemistry 34, 15395-15406.

Cohen P., Alessi D.R. and Cross D.A. (1997). PDK1, one of the missing links in insulin signal transduction? FEBS Lett. 410, 3-10.

Cole A.A. and Wezeman F.H. (1985). Perivascular cells in cartilage canals of the developing mouse epiphysis. Am. J. Anat. 174, 119-129.

Cole A.A. and Wezeman F.H. (1987). Morphometric analysis of cartilage canals in the developing mouse epiphysis. Acta Anat. 128, 93-97.

Craik J.D., Stewart M. and Cheeseman C.I. (1995). GLUT-3 (brain-type) glucose transporter polypeptides in human blood platelets. Thromb. Res. 79, 461-469.

Czech M.P. and Corvera S. (1999). Signalling mechanisms that regulate glucose transport. J. Biol. Chem. 274, 1865-1868.

Dawson P.A., Mychaleckyj J.C., Fossey S.C., Mihic S.J., Craddock A.L. and Bowden D.W. (2001). Sequence and functional analysis of GLUT10: a glucose transporter in the type 2-diabetes-linked region of chromosome 20q12-13.1. Mol. Genet. Metab. 74, 186-199.

Delgado-Baeza E., Nieto-Chaguaceda A., Miralles-Flores C. and Santos-Alvarez I. (1992). Cartilage canal growth: experimental approach in the rat tibia. Acta Anat. (Basel). 145, 143-148.

De Meyts P., Wallach B., Christoffersen C.T., Urso B., Gronskov K., Latus L.J., Yakushiji F., Ilondo M.M. and Shymko R.M. (1994). The insulin-like growth factor-I receptor structure, ligand-binding mechanism and signal transduction. Horm. Res. 42, 152-169.

Del Rey A. and Besedovsky H. (1987). Interleukin 1 affects glucose homeostasis. Am. J. Physiol. 253, R794-R798.

Denko C.W. and Malemud C.J. (1999). Metabolic disturbances and synovial joint responses in osteoarthritis. Front. Biosci. 4, D686-D693.

Denko C.W., Boja B. and Moskowitz R.W. (1994). Growth promoting peptides in osteoarthritis and diffuse idiopathic skeletal hyperostosis--insulin, insulin-like growth factor-I, growth hormone. J. Rheumatol. 21, 1725-1730.

Dienel G.A., Cruz N.F., Mori K., Holden J.E. and Sokoloff L. (1991). Direct measurement of the lambda of the lumped constant of the deoxyglucose method in rat brain: determination of lambda and lumped constant from tissue glucose concentration or equilibrium brain/plasma distribution ratio for methylglucose. J. Cereb. Blood Flow Metab. 11, 25-34.

Dimmer K.S., Friedrich B., Lang F., Deitmer J.W. and Broer S. (2000). The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. Biochem. J. 350, 219-227.

Dixon S.J. and Wilson J.X. (1992). Transforming growth factor-ß stimulates ascorbate transport activity in osteoblastic cells. Endocrinology 130, 484–489.

Doege H., Bocianski A., Joost H-G. and Schürmann A. (2000a). Activity and genomic organization of human glucose transporter 9 (GLUT9), a novel member of the family of sugar transport facilitators predominantly expressed in brain and leukocytes. Biochem. J. 350, 771-776.

Doege H., Schürmann A., Bahrenberg G., Brauers A. and Joost H-G. (2000b). Glucose transporter 8 (GLUT): A novel sugar transport facilitator with glucose transport activity. J. Biol. Chem. 275, 16275-16280.

Doege H., Bocianski A., Scheepers A., Axer H., Eckel J., Joost H-G. and Schürmann. A. (2001). Characterization of glucose transporter 10 (GLUT10), a novel heart and skeletal muscle-specific sugar transport facilitator. Diabetes 50, A277-A278.

Doege H., Bocianski A., Scheepers A., Axer H., Eckel J., Joost H-G. and Schürmann A. (2001). Characterization of human glucose transporter (GLUT) 11 (encoded by SLC2A11), a novel sugar-transport facilitator specifically expressed in heart and skeletal muscle. Biochem J. 359, 443-449.

Doré S., Pelletier J.B., DiBattista J.A., Tardiff G., Brazeau P. and Martel-Pelletier J. (1994). Human osteoarthritic chondrocytes possess an increased number of insulin-like growth factor 1 binding sites but are unresponsive to its stimulation. Possible role of IGF-1-binding proteins. Arthritis Rheum. 37, 253-263.

Dumaine R. and Kirsch G.E. (1998). Mechanism of lidocaine block of late current in long Q-T mutant Na+ channels. Am. J. Physiol. 274, H477-H487.

Figenschau Y., Knutsen G., Shahazeydi S., Johansen O. and Sveinbjornsson B. (2001). Human articular chondrocytes express functional leptin receptors. Biochem. Biophys. Res. Commun. 287, 190-197.

Fillmore C.M., Bartoli L., Bach R. and Park Y. (1999). Nutrition and dietary supplements. Phys. Med. Rehabil. Clin. N. Am. 10, 673-703.

Forster C., Rucker M., Shakibaei M., Baumann-Wilschke I., Vormann J. and Stahlmann R. (1998). Effects of fluoroquinolones and magnesium deficiency in murine limb bud cultures. Arch. Toxicol. 72, 411-419.

Franceschi R.T. (1992). The role of ascorbic acid in mesenchymal differentiation. Nutr. Rev. 50, 65-70.

Franceschi R.T. and Iyer B.S. (1992). Relationship between collagen synthesis and expression of the osteoblast phenotype in MC3T3-E1 cells. J. Bone Miner. Res. 7, 235-246.

Franceschi R.T., Wilson J.X. and Dixon S.J. (1995). Requirement for Na^+-dependent ascorbic acid transport in osteoblast function. Am. J. Physiol. 268, C1430–C1439.

Fukumoto H., Seino S., Imura H., Seino Y., Eddy R.L., Fukushima Y., Byers M.G., Shows T.B. and Bell G.I. (1988). Sequence, tissue distribution, and chromosomal localization of mRNA encoding a

human glucose transporter-like protein. Proc Natl Acad Sci USA 85, 5434-5438.

Fukumoto H., Kayano T., Buse J.B., Edwards Y., Pilch P.F., Bell G.I. and Seino S. (1989). Cloning and characterization of the major insulin-responsive glucose transporter expressed in human skeletal muscle and other insulin-responsive tissues. J. Biol. Chem. 264, 7776-7779.

Ganey T.M., Love S.M. and Ogden J.A. (1992). Development of vascularization in the chondroepiphysis of the rabbit. J. Orthop. Res. 10, 496-510.

Ghosh P. (1999). The pathobiology of osteoarthritis and the rationale for the use of pentosan polysulfate for its treatment. Semin. Arthritis Rheum. 28, 211-267.

Goldring M.B. (1999). The role of cytokines as inflammatory mediators in osteoarthritis: lessons from animal models. Connect. Tissue Res. 40, 1-11.

Goldring M.B. (2000a). The role of the chondrocyte in osteoarthritis. Arthritis Rheum. 43, 1916-1926.

Goldring M.B. (2000b). Osteoarthritis and cartilage: the role of cytokines. Curr. Rheumatol. Rep. 2, 459-465.

Gould G.W., Thomas H.M., Jess T.J. and Bell G.I. (1991). Expression of human glucose transporters in *Xenopus* oocytes: kinetic characterization and substrate specificities of the erythrocyte, liver, and brain isoforms. Biochemistry 30, 5139-5145.

Grandolfo M., D'Andrea P., Martina M., Ruzzier F. and Vittur F. (1992). Calcium-activated potassium channels in chondrocytes. Biochem. Biophys. Res. Commun. 182, 1429-1434.

Grant A.O. and Wendt D.J. (1992). Block and modulation of cardiac Na^+ channels by antiarrhythmic drugs, neurotransmitters and hormones. Trends Pharmacol. Sci. 13, 352-358.

Gruetter R., Novotny E.J., Boulware S.D., Rothman D.L., Mason G.F., Shulman G.I., Shulman R.G. and Tamborlane W.V. (1992). Direct measurement of brain glucose concentrations in humans by 13C NMR spectroscopy. Proc. Natl. Acad. Sci. USA 89, 1109-1112.

Grigoriadis A.E., Heersche J.N. and Aubin J.E. (1990). Continuously growing bipotential and monopotential myogenic, adipogenic, and chondrogenic subclones isolated from the multipotential RCJ 3.1 clonal cell line. Dev. Biol. 142, 313-318.

Guilak F. (2000). The deformation behavior and viscoelastic properties of chondrocytes in articular cartilage. Biorheology 37, 27-44.

Haber R.S., Weinstein S.P., O'Boyle E. and Morgello S. (1993). Tissue distribution of the human GLUT3 glucose transporter. Endocrinology 132, 2538-2543.

Halestrap A.P. and Price N.T. (1999). The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. Biochem. J. 343, 281-299.

Hamrahian A.H., Zhang J.Z., Elkhairi F.S., Prasad R. and Ismail-Beigi F. (1999). Activation of Glut1 glucose transporter in response to inhibition of oxidative phosphorylation. Arch. Biochem. Biophys. 368, 375-379.

Harik S.I., Behmand R.A. and LaManna J.C. (1994). Hypoxia increases glucose transport at blood-brain barrier in rats. J. Appl. Physiol. 77, 896-901.

Haudenschild D.R., McPherson J.M., Tubo R. and Binette F. (2001). Differential expression of multiple genes during articular chondrocyte redifferentiation. Anat. Rec. 263, 91-98.

Haugen M., Homme K.A., Reigstad A. and Teigland J. (1999). Assessment of nutritional status in patients with rheumatoid arthritis and osteoarthritis undergoing joint replacement surgery. Arthritis Care Res. 12, 26-32.

Hediger M.A., Turk E. and Wright E.M. (1989). Homology of the human intestinal Na+/glucose and Escherichia coli Na+/proline cotransporters. Proc. Natl. Acad. Sci. USA. 86, 5748-5752.

Hernvann A., Aussel C., Cynober L., Moatti N. and Ekindjian O.G. (1992). IL-1 a strong mediator for glucose uptake by rheumatoid and non-rheumatoid cultured human synoviocytes. FEBS Lett. 303, 77-80.

Hernvann A., Jaffray P., Hilliquin P., Cazalet C., Menkes C.-J. and Ekindjian. O.G. (1996). Interleukin-1-mediated glucose uptake by chondrocytes: inhibition by cortisol. Osteoarthritis Cartilage 4, 139-142.

Holman G.D. and Kasuga M. (1997). From receptor to transporter: insulin signalling to glucose transport. Diabetologia. 40, 991-1003.

Ibberson M., Uldry M. and Thorens B. (2000). GLUTX1, a novel mammalian glucose transporter expressed in the central nervous system and insulin sensitive tissues. J. Biol. Chem. 275, 4607-4612.

Iannotti J.P. (1990). Growth plate physiology and pathology. Orthop. Clin. North Am. 21, 1-17.

Ishihara H. and Urban J.P. (1999). Effects of low oxygen concentrations and metabolic inhibitors on proteoglycan and protein synthesis rates in the intervertebral disc. J. Orthop. Res. 17, 829-835.

James D.E., Strube M. and Mueckler M. (1989). Molecular cloning and characterization of an insulin-regulatable glucose transporter. Nature 338, 83-87.

Johnson K.A., Hulse D.A., Hart R.C., Kochevar D. and Chu Q. (2001). Effects of an orally administered mixture of chondroitin sulfate, glucosamine hydrochloride and manganese ascorbate on synovial fluid chondroitin sulfate 3B3 and 7D4 epitope in a canine cruciate ligament transection model of osteoarthritis. Osteoarthritis Cartilage 9, 14-21.

Jones N.M. and Bergeron M. (2001). Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. J. Cereb. Blood Flow Metab. 21, 1105-1114.

Joost H-G. and Thorens B. (2001). The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members. Mol. Membr. Biol. 18, 247–256.

Joost H-G., Bell G.I., Best J.D., Birnbaum M.J., Charron M.J., Chen Y.T., Doege H., James D.E., Lodish H.F., Moley K.H., Moley J.F., Mueckler M., Rogers S., Schurmann A., Seino S. and Thorens B. (2002). Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am. J. Physiol. 282, E974-E976.

Kayano T., Fukumoto H., Eddy R.L., Fan Y.S., Byers M.G., Shows T.B. and Bell G.I. (1988). Evidence for a family of human glucose transporter-like proteins. Sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. J. Biol. Chem. 263, 15245-15248.

Kayano T., Burant C.F., Fukumoto H., Gould G.W., Fan Y., Eddy R.L., Byers M.G., Seino S. and Bell G.I. (1990). Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J. Biol. Chem. 265, 13267-13282.

Kealy R.D., Lawler D.F., Ballam J.M., Lust G., Smith G.K., Biery D.N. and Olsson S.E. (1997). Five-year longitudinal study on limited food consumption and development of osteoarthritis in coxofemoral joints of dogs. J. Am. Vet. Med. Assoc. 210, 222-225.

Kelley K.M., Johnson T.R., Ilan J. and Moskowitz R.W. (1999). Glucose regulation of the IGF response system in chondrocytes: induction of an IGF-I-resistant state. Am. J. Physiol. 276, R1164-R1171.

Kim J.J. and Conrad H.E. (1976). Kinetics of mucopolysaccharide and glycoprotein synthesis by chick embryo chondrocytes. Effect of D-glucose concentration in the culture medium. J. Biol. Chem. 251, 6210-6217.

Kirsten R., Nelson K., Kirsten D. and Heintz B. (1998). Clinical pharmacokinetics of vasodilators. Part I. Clin. Pharmacokinet. 34, 457-482.

Kumar S., Connor J.R., Dodds R.A., Halsey W., Van Horn M., Mao J., Sathe G., Mui P., Agarwal P., Badger A.M., Lee J.C., Gowen M. and Lark M.W. (2001). Identification and initial characterization of 5000 expressed sequenced tags (ESTs) each from adult human normal and osteoarthritic cartilage cDNA libraries. Osteoarthritis Cartilage 9, 641-653.

Kume K., Satomura K., Nishisho S., Kitaoka E., Yamanouchi K., Tobiume S. and Nagayama M. (2002). Potential role of leptin in endochondral ossification. J. Histochem. Cytochem. 50, 159-170.

Ku Tai P-K., Liao J-F., Hossler P.A., Castor C.W. and Carter-Su C. (1992). Regulation of glucose transporters by connective tissue activating peptide-III isoforms. J. Biol. Chem. 267, 19579-19586.

Lant A. (1986). Diuretic drugs. Progress in clinical pharmacology. Drugs. 31, 40-55.

Lant A.F. (1987). Evolution of diuretics and ACE inhibitors, their renal and antihypertensive actions--parallels and contrasts. Br. J. Clin. Pharmacol. 23, 27S-41S.

Lee R.B. and Urban J.P. (1997). Evidence for a negative Pasteur effect in articular cartilage. Biochem. J. 321, 95-102.

Levick J.R. (1995). Microvascular architecture and exchange in synovial joints. Microcirculation 2, 217-233.

Liang W.J., Johnson D. and Jarvis S.M. (2001). Vitamin C transport systems of mammalian cells. Mol. Membr. Biol. 18, 87-95.

Lioté F. and Orcel P. (2000). Osteoarticular disorders of endocrine origin. Baillieres Best Pract. Res. Clin. Rheumatol. 14, 251-276.

Lisinski I., Schurmann A., Joost H-G., Cushman S.W. and Al-Hasani H. (2001). Targeting of GLUT6 (formerly GLUT9) and GLUT8 in rat adipose cells. Biochem. J. 358, 517-522.

Maher F. and Simpson I.A. (1994). The GLUT3 glucose transporter is the predominant isoform in primary cultured neurons: assessment by biosynthetic and photoaffinity labelling. Biochem. J. 301, 379-384.

Maher F., Vannucci S.J. and Simpson I.A. (1994). Glucose transporter proteins in brain. FASEB J. 8, 1003-1011.

Maher F., Davies-Hill T.M. and Simpson I.A. (1996). Substrate specificity and kinetic parameters of GLUT3 in rat cerebellar granule neurons. Biochem. J. 315, 827-831.

Marette A., Burdett E., Douen A., Vranic M. and Klip A. (1992). Insulin induces the translocation of GLUT4 from a unique intracellular organelle to transverse tubules in rat skeletal muscle. Diabetes 41, 1562-1569.

Marette A., Dimitrakoudis D., Shi Q., Rodgers C.D., Klip A. and Vranic M. (1999). Glucose rapidly decreases plasma membrane GLUT4 content in rat skeletal muscle. Endocrine 10, 13-18.

Martel-Pelletier J. (1998). Pathophysiology of osteoarthritis. Osteoarthritis Cartilage 6, 374-376.

Mason R.M., Kimura J.H. and Hascall V.C. (1982). Biosynthesis of hyaluronic acid in cultures of chondrocytes from Swarm rat chondrosarcoma. J. Biol. Chem. 257, 2236-2245.

McAlindon T. and Felson D.T. (1997). Nutrition: risk factors for osteoarthritis. Ann. Rheum. Dis. 56, 397-400.

McAlindon T.E., LaValley M.P., Gulin J.P. and Felson D.T. (2000). Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. JAMA 283, 1469-1475.

McVie-Wylie A.J., Lamson D.R. and Chen Y.T. (2001). Molecular cloning of a novel member of the GLUT family of transporters, SLC2A10 (GLUT10), localized on chromosome 20q13.1: A candidate gene for NIDDM susceptibility. Genomics 72, 113-117.

Meacock S.C.R., Bodmer J.L. and Billingham M.E.J. (1990). Experimental osteoarthritis in guinea pigs. J. Exp. Pathol. 71, 279-293.

Mobasheri A. (2002). Role of chondrocyte death and hypocellularity in ageing human articular cartilage and the pathogenesis of osteoarthritis. Medical Hypotheses 58, 193-197

Mobasheri A., Hall A.C., Urban J.P., France S.J. and Smith A.L. (1997a). Immunologic and autoradiographic localisation of the Na+, K+-ATPase in articular cartilage: upregulation in response to changes in extracellular Na+ concentration. Int. J. Biochem. Cell Biol. 29, 649-657.

Mobasheri A., Errington R.J., Golding S., Hall A.C. and Urban J.P. (1997b). Characterization of the Na^+, K^+-ATPase in isolated bovine articular chondrocytes; molecular evidence for multiple alpha and beta isoforms. Cell Biol. Int. 21, 201-212.

Mobasheri A., Mobasheri R., Francis M.J.O., Trujillo E., Alvarez De La Rosa D. and Martín-Vasallo P. (1998). Ion transport in chondrocytes: membrane transporters involved in intracellular ion homeostasis and the regulation of cell volume, free [Ca^{2+}] and pH. Histol. Histopathol. 13, 893-910.

Mobasheri A. and Martín-Vasallo P. (1999). Epithelial sodium channels in skeletal cells; a role in mechanotransduction? Cell Biol. Int. 23, 237-240.

Mobasheri A., Neama G., Bell S. and Carter S.D. (2001). Molecular evidence for two glucose transporters (GLUT1 and GLUT3) in adult human articular cartilage. Int. J. Exp. Pathol. 82, A14.

Mobasheri A., Neama G., Bell S., Richardson S. and Carter S.D. (2002a). Human articular chondrocytes express three facilitative glucose transporter isoforms: GLUT1, GLUT3 and GLUT9. Cell Biol. Int. 26, 297-300

Mobasheri A., Carter S.D., Martín-Vasallo P. and Shakibaei M. (2002b). Integrins and stretch activated ion channels; putative components of functional cell surface mechanoreceptors in articular chondrocytes. Cell Biol. Int. 26, 1-18.

Mobasheri A., Richardson S., Neama G., Bell S. and Carter S.D. (2002c). Expression of facilitative glucose transporters 1, 3, 5, 9, 10 and 11 in human articular cartilage. Pflügers Archiv. (Eur. J. Physiol.) 443 Suppl. S240.

Mueckler M. (1994). Facilitative glucose transporters. Eur. J. Biochem. 219, 713-725.

Mueckler M., Caruso C., Baldwin S.A., Panico M., Blench I., Morris H.R., Allard W.J., Lienhard G.E. and Lodish H.F. (1985). Sequence and structure of a human glucose transporter. Science 229, 941-945.

Munoz-Torres M. (1996). Bone mineral density measured by dual x-ray absorptiometry in Spanish patients with insulin-dependent diabetes mellitus. Calcif. Tissue Int. 58, 316-319.

Munteanu S.E., Ilic M.Z. and Handley C.J. (2000). Calcium pentosan polysulfate inhibits the catabolism of aggrecan in articular cartilage explant cultures. Arthritis Rheum. 43, 2211-2218.

Neama G., Bell S., Richardson S., Carter S. and Mobasheri A. (2001). Molecular evidence for GLUT1 and GLUT3 in human articular cartilage and immortalized human chondrocytes: changes in GLUT expression levels in long-term alginate culture J. Physiol. 535P, 42P-43P.

Ohara H., Tamayama T., Maemura K., Kanbara K., Hayasaki H., Abe M. and Watanabe M. (2001). Immunocytochemical demonstration of glucose transporters in epiphyseal growth plate chondrocytes of young rats in correlation with autoradiographic distribution of 2-deoxyglucose in chondrocytes. Acta Histochem. 103, 365-378.

Okma-Keulen P. and Hopman-Rock M. (2001). The onset of generalized osteoarthritis in older women: a qualitative approach. Arthritis Rheum. 45, 183-190.

Otte P. (1991). Basic cell metabolism of articular cartilage. Manometric studies. Z. Rheumatol. 50, 304-312.

Pessin J.E., Thurmond D.C., Elmendorf J.S., Coker K.J. and Okada S. (1999). Molecular basis of insulin-stimulated GLUT4 vesicle trafficking. Location! Location! Location! J. Biol. Chem. 274, 2593-2596.

Phay J.E., Hussain H.B. and Moley J.F. (2000). Cloning and expression analysis of a novel member of the facilitative glucose transporter family, SLC2A9 (GLUT9). Genomics 66, 217-220.

Pickart L. and Thaler M.M. (1980). Responses of rat and chick chondrocytes and rate hepatoma cells to plasma fractions with insulin-like and growth-promoting activities. Biochim. Biophys. Acta. 632, 112-120.

Pilegaard H., Terzis G., Halestrap A. and Juel C. (1999). Distribution of the lactate/H^+ transporter isoforms MCT1 and MCT4 in human skeletal muscle. Am. J. Physiol. 276, E843-E848.

Pritcherd J.J. (1952). A cytological and histochemical study of bone and cartilage formation. J. Anat. 8, 259-277

Qutob S., Dixon S.J. and Wilson J.X. (1998). Insulin stimulates vitamin C recycling and ascorbate accumulation in osteoblastic cells. Endocrinology 139, 51-56.

Rauchman M.I., Wasserman J.C., Cohen D.M., Perkins D.L., Hebert S.C., Milford E. and Gullans S.R. (1992). Expression of GLUT-2 cDNA in human B lymphocytes: analysis of glucose transport using flow cytometry. Biochim. Biophys. Acta. 1111, 231-238.

Rea S. and James D.E. (1997). Moving GLUT4: the biogenesis and trafficking of GLUT4 storage vesicles. Diabetes 46, 1667-1677.

Reginster J.Y., Gillot V., Bruyere O. and Henrotin Y. (2000). Evidence of nutriceutical effectiveness in the treatment of osteoarthritis. Current Rheumatol. Rep. 2, 472-477.

Reginster J.Y., Deroisy R., Rovati L.C., Lee R.L., Lejeune E., Bruyere O., Giacovelli G., Henrotin Y., Dacre J.E. and Gossett C. (2001). Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo controlled clinical trial. Lancet. 357, 251-256.

Richardson D.C. and Zentek J. (1998). Nutrition and osteochondrosis. Vet. Clin. North Am. Small Anim. Pract. 28, 115-135.

Richardson D.W. and Dodge G.R. (2000). Effects of interleukin-1ß and tumour necrosis factor-alpha on expression of matrix-related genes by cultured equine articular chondrocytes. Am. J. Vet. Res. 61, 624-630.

Robbins J.R., Thomas B., Tan L., Choy B., Arbiser J.L., Berenbaum F., Goldring M.B. (2000). Immortalized human adult articular chondrocytes maintain cartilage-specific phenotype and responses to interleukin-1beta. Arthritis Rheum. 43, 2189-2201.

Rogers S, James DE, and Best JD. (1998). Identification of novel facilitative transporter like protein-GLUT8. Diabetes 47 (Suppl. 1), A45.

Rogers S., Macheda M.L., Docherty S.E., Carty M.D., Henderson M.A., Soeller W.C., Gibbs E.M., James D.E. and Best J.D. (2002). Identification of a novel glucose transporter-like protein-GLUT-12. Am. J. Physiol. 282, E733-E738

Rosenbloom A.L. and Silverstein J.H. (1996). Connective tissue and joint disease in diabetes mellitus. Endocrinol. Metab. Clin. North Am. 25, 473-483.

Rumsey S.C., Kwon O., Xu G.W., Burant C.F., Simpson I. and Levine M. (1997). Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. J. Biol. Chem. 272, 18982-18989.

Rumsey S.C., Welch R.W., Garraffo H.M., Ge P., Lu S.F., Crossman A.T., Kirk K.L. and Levine M. (1999). Specificity of ascorbate analogs for ascorbate transport. Synthesis and detection of [(^{125})I]6-deoxy-6-iodo-L-ascorbic acid and characterization of its ascorbate-specific transport properties. J. Biol. Chem. 274, 23215-23222.

Rumsey S.C., Daruwala R., Al-Hasani H., Zarnowski M.J., Simpson I.A. and Levine M. (2000). Dehydroascorbic acid transport by GLUT4 in Xenopus oocytes and isolated rat adipocytes. J. Biol. Chem. 275, 28246-28253.

Sachs G. (2001). Improving on PPI-based therapy of GORD. Eur. J. Gastroenterol. Hepatol. 13 (Suppl 1), S35-S41.

Sachs G. and Wallmark B. (1989). The gastric H^+,K^+-ATPase: the site of action of omeprazole. Scand. J. Gastroenterol. Suppl. 166, 3-11.

Sasaki T., Minoshima S., Shiohama A., Shintani A., Shimizu A., Asakawa S., Kawasaki K. and Shimizu N. (2001). Molecular cloning of a member of the facilitative glucose transporter gene family GLUT11 (SLC2A11) and identification of transcription variants. Biochem. Biophys. Res. Commun. 289, 1218-1224.

Scheepers A., Doege H., Joost H-G. and Schurmann A. (2001). Mouse GLUT8: genomic organization and regulation of expression in 3T3-L1 adipocytes by glucose. Biochem. Biophys. Res. Commun. 288, 969-974.

Schuit F.C. (1996). Factors determining the glucose sensitivity and glucose responsiveness of pancreatic beta cells. Horm. Res. 46, 99-106.

Schwartz E.R., Oh W.H. and Leveille C.R. (1981). Experimentally induced osteoarthritis in guinea pigs. Arthritis Rheum. 24, 1345-1355.

Setnikar I. (2001). Glucosamine for osteoarthritis. Sound science might have helped avoid confusion. BMJ. 323, 1003-1004.

Setnikar I. and Rovati L.C. (2001). Absorption, distribution, metabolism and excretion of glucosamine sulfate. A review. Arzneimittelforschung 51, 699-725.

Slater M.R., Scarlett J.M, Donoghue S., Kaderly R.E., Bonnett B.N, Cockshutt J. and Erb H.N. (1992). Diet and exercise as potential risk factors for osteochondritis dissecans in dogs. Am. J. Vet. Res. 53, 2119-2124.

Shakibaei M., Kociok K., Forster C., Vormann J., Gunther T., Stahlmann R. and Merker H.J. (1996). Comparative evaluation of ultrastructural changes in articular cartilage of ofloxacin-treated and magnesium-deficient immature rats. Toxicol. Pathol. 24, 580-587.

Shakibaei M. and Mobasheri A. (2000). Sodium pumps, epithelial sodium channels and voltage-gated calcium channels colocalize with b1-integrins in mouse limb-bud chondrocytes. J. Physiol. (Lond.). 527P, 111P.

Shepherd P.R., Gould G.W., Colville C.A., McCoid S.C., Gibbs E.M. and Kahn BB. (1992). Distribution of GLUT3 glucose transporter protein

in human tissues. Biochem. Biophys. Res. Commun. 188, 149-54.

Shikhman A.R., Kuhn K., Alaaeddine N. and Lotz M. (2001a). N-acetylglucosamine prevents IL-1--mediated activation of human chondrocytes. J. Immunol. 166, 5155-5160.

Shikhman A.R., Brinson D., Valbracht J. and Lotz M. (2001b). Cytokine regulation of facilitated glucose transport in human articular chondrocytes. J. Immunol. 167, 7001-7008.

Skawina A., Litwin J.A., Gorczyca J. and, Miodonski A.J. (1994). Blood vessels in epiphyseal cartilage of human fetal femoral bone: a scanning electron microscopic study of corrosion casts. Anat. Embryol. (Berl). 189, 457-462.

Stahlmann R., Forster C., Shakibaei M., Vormann J., Gunther T. and Merker H.J. (1995). Magnesium deficiency induces joint cartilage lesions in juvenile rats which are identical to quinolone-induced arthropathy. Antimicrob. Agents Chemother. 39, 2013-2018.

Stahlmann R., Kuhner S., Shakibaei M., Flores J., Vormann J. and van Sickle D.C. (2000). Effects of magnesium deficiency on joint cartilage in immature beagle dogs: immunohistochemistry, electron microscopy, and mineral concentrations. Arch. Toxicol. 73, 573-580.

Stavrou S. and Kleinberg D.L. (2001). Rheumatic manifestations of pituitary tumors. Curr. Rheumatol. Rep. 3, 459-463.

Sugimoto T., Nakada M., Fukase M., Imai Y., Kinoshita Y. and Fujita T. (1986). Effects of ascorbic acid on alkaline phosphatase activity and hormone responsiveness in the osteoblastic osteosarcoma cell line UMR-106. Calcif. Tissue Int. 39, 171-174.

Studer R.K., Georgescu H.I., Miller L.A. and Evans C.H. (1999). Inhibition of transforming growth factor beta production by nitric oxide-treated chondrocytes: implications for matrix synthesis. Arthritis Rheum. 42, 248-257.

Sweeney C., Mackintosh D. and Mason R.M. (1993). UDP-sugar metabolism in Swarm rat chondrosarcoma chondrocytes. Biochem. J. 290, 563-570.

Taskiran D., Stefanovic-Racic M., Georgescu H. and Evans C. (1994). Nitric-oxide mediates suppression of cartilage proteoglycan synthesis by Interleukin-1. Biochem. Biophys. Res. Commun. 200, 142-148.

Thomson A.B. and Wild G. (1997a). Adaptation of intestinal nutrient transport in health and disease. Part I. Dig. Dis. Sci. 42, 453-469.

Thomson A.B. and Wild G. (1997b). Adaptation of intestinal nutrient transport in health and disease. Part II. Dig. Dis. Sci. 42, 470-488.

Thorens B. (2001). GLUT2 in pancreatic and extra-pancreatic gluco-detection (review). Mol. Membr. Biol. 18, 265-273.

Torzilli P.A., Arduino J.M., Gregory J.D. and Bansal M. (1997). Effect of proteoglycan removal on solute mobility in articular cartilage. J. Biomech. 30, 895-902.

Torzilli P.A., Grande D.A. and Arduino J.M. (1998). Diffusive properties of immature articular cartilage. J. Biomed. Mater. Res. 40, 132-138.

Trippel S.B. (1995). Growth factor actions on articular cartilage. J. Rheumatol. Suppl. 43, 129-132.

Trujillo E., Alvarez de la Rosa D., Mobasheri A., Avila J., Gonzalez T. and Martin-Vasallo P. (1999a). Sodium transport systems in human chondrocytes. I. Morphological and functional expression of the Na+, K+-ATPase alpha and beta subunit isoforms in healthy and arthritic chondrocytes. Histol. Histopathol. 14, 1011-1022.

Trujillo E., Alvarez de la Rosa D., Mobasheri A., Gonzalez T., Canessa C.M. and Martin-Vasallo P. (1999b). Sodium transport systems in human chondrocytes. II. Expression of ENaC, $Na^+/K^+/2Cl^-$ cotransporter and Na^+/H^+ exchangers in healthy and arthritic chondrocytes. Histol. Histopathol. 14, 1023-1031.

Tsukaguchi H., Tokui T., Mackenzie B., Berger U.V., Chen X.Z., Wang Y., Brubaker R.F. and Hediger M.A. (1999). A family of mammalian Na^+-dependent L-ascorbic acid transporters. Nature 399, 70-75.

Uldry M., Ibberson M., Riederer B., Chatton J.Y., Horisberger J.D. and Thorens B. (2001). Identification of a novel H+-myo-inositol symporter expressed predominantly in the brain. EMBO J. 20, 4467-4477.

Underwood L.E. (1996). Nutritional regulation of IGF-I and IGFBPs. J. Pediatr. Endocrinol. Metab. 9, 303-312.

van den Berg W.B. (1999). The role of cytokines and growth factors in cartilage destruction in osteoarthritis and rheumatoid arthritis. Z. Rheumatol. 58, 136-141.

Vannucci R.C. and Vannucci S.J. (2000). Glucose metabolism in the developing brain. Semin. Perinatol. 24, 107-115.

Vannucci S.J. (1994). Developmental expression of GLUT1 and GLUT3 glucose transporters in rat brain. J. Neurochem. 62, 240-246.

Vannucci S.J., Seaman L.B., Brucklacher R.M. and Vannucci R.C. (1994a). Glucose transport in developing rat brain: glucose transporter proteins, rate constants and cerebral glucose utilization. Mol. Cell. Biochem. 140, 177-184.

Vannucci S.J., Maher F., Koehler E. and Simpson I.A. (1994b). Altered expression of GLUT-1 and GLUT-3 glucose transporters in neurohypophysis of water-deprived or diabetic rats. Am. J. Physiol. 267, E605-E611.

Vannucci S.J., Seaman L.B. and Vannucci R.C. (1996). Effects of hypoxia-ischemia on GLUT1 and GLUT3 glucose transporters in immature rat brain. J. Cereb. Blood Flow Metab. 16, 77-81.

Vannucci S.J., Maher F., Simpson I.A. (1997). Glucose transporter proteins in brain: delivery of glucose to neurons and glia. Glia. 21, 2-21.

Vannucci S.J., Reinhart R., Maher F., Bondy C.A., Lee W.H., Vannucci R.C. and Simpson I.A. (1998). Alterations in GLUT1 and GLUT3 glucose transporter gene expression following unilateral hypoxia-ischemia in the immature rat brain. Brain Res. Dev. Brain Res. 107, 255-264.

Vannucci S.J., Rutherford T., Wilkie M.B., Simpson I.A. and Lauder J.M. (2000). Prenatal expression of the Glut4 glucose transporter in the mouse. Dev. Neurosci. 22, 274-282.

Vera J.C., Rivas C.I., Velasquez F.V., Zhang R.H., Concha I.I. and Golde D.W. (1995). Resolution of the facilitated transport of dehydroascorbic acid from its intracellular accumulation as ascorbic acid. J. Biol. Chem. 270, 23706-23712.

Vera J.C., Reyes A.M., Velasquez F.V., Rivas C.I., Zhang R.H., Strobel P., Slebe J.C., Nunez-Alarcon J. and Golde D.W. (2001). Direct inhibition of the hexose transporter GLUT1 by tyrosine kinase inhibitors. Biochemistry 40, 777-790.

Verhaeghe J., Suiker A.M., Einhorn T.A., Geusens P., Visser W.J., Van Herck E., Van Bree R., Magitsky S. and Bouillon R. (1994a). Brittle bones in spontaneously diabetic female rats cannot be predicted by bone mineral measurements: studies in diabetic and ovariectomized rats. J. Bone Miner. Res. 9, 1657-1667.

Verhaeghe J., Allewaert K., Van Herck E., Van Bree R., Van Assche F.A. and Bouillon R. (1994b). 1,25-Dihydroxyvitamin D3 and osteocalcin in maternal and fetal guinea pigs. Bone Miner. 26, 261-273.

von der Mark K., Kirsch T., Nerlich A., Kuss A., Weseloh G., Gluckert K. and Stoss H. (1992). Type X collagen synthesis in human osteoarthritic cartilage. Indication of chondrocyte hypertrophy. Arthritis Rheum. 35, 806-811.

von Grumbckow L., Elsner P., Hellsten Y., Quistorff B. and Juel C. (1999). Kinetics of lactate and pyruvate transport in cultured rat myotubes. Biochim. Biophys. Acta. 1417, 267-275.

Wang J., Zhou J. and Bondy C.A. (1999). Igf1 promotes longitudinal bone growth by insulin-like actions augmenting chondrocyte hypertrophy. FASEB J. 13, 1985-1990.

Wang X.T., Nagaba S., Nagaba Y., Leung S.W., Wang J., Qiu W., Zhao P.L. and Guggino S.E. (2000). Cardiac L-type calcium channel alpha 1-subunit is increased by cyclic adenosine monophosphate: messenger RNA and protein expression in intact bone. J. Bone Miner. Res. 15, 1275-1285.

Williams J.S. Jr., Bush-Joseph C.A. and Bach B.R. Jr. (1998). Osteochondritis dissecans of the knee. Am. J. Knee Surg. 11, 221-232.

Wilson J.X. and Dixon S.J. (1995). Ascorbate concentration in osteoblastic cells is elevated by transforming growth factor-ß. Am. J. Physiol. 268, E565–E571

Wilson C.M., Mitsumoto Y., Maher F. and Klip A. (1995). Regulation of cell surface GLUT1, GLUT3, and GLUT4 by insulin and IGF-I in L6 myotubes. FEBS Lett. 368, 19–22.

Wilhelmi G. (1993a). Potential effects of nutrition including additives on healthy and arthrotic joints. I. Basic dietary constituents. Z. Rheumatol. 52, 174-179.

Wilhelmi G. (1993b). Potential influence of nutrition with supplements on healthy and arthritic joints. II. Nutritional quantity, supplements, contamination. Z. Rheumatol. 52, 191-200.

Wolf G. (1996). The mechanism of uptake of ascorbic acid into osteoblasts and leukocytes. Nutr. Rev. 54, 150-152.

Woodard J.C., Becker H.N. and Poulos P.W. Jr. (1987a). Effect of diet on longitudinal bone growth and osteochondrosis in swine. Vet. Pathol. 24, 109-117.

Woodard J.C., Becker H.N. and Poulos P.W. Jr. (1987b). Articular cartilage blood vessels in swine osteochondrosis. Vet. Pathol. 24, 118-123.

Wozney J.M. (1995). The potential role of bone morphogenetic proteins in periodontal reconstruction. J. Periodontol. 66, 506-510.

Wright E.M., Hager K.M. and Turk E. (1992). Sodium cotransport proteins. Curr. Opin. Cell Biol. 4, 696-702.

Yamaguchi T., Hayashi K., Tayama N. and Sugioka Y. (1990). The role of cartilage canals: experimental study using rabbits' femoral heads. Nippon Seikeigeka Gakkai Zasshi 64, 1105-1110.

Yamakura T., Bertaccini E., Trudell J.R. and Harris R.A. (2001). Anesthetics and ion channels: molecular models and sites of action. Annu. Rev. Pharmacol. Toxicol. 41, 23-51.

Yosimichi G., Nakanishi T., Nishida T., Hattori T., Takano-Yamamoto T. and Takigawa M. (2001). CTGF/Hcs24 induces chondrocyte differentiation through a p38 mitogen-activated protein kinase (p38MAPK), and proliferation through a p44/42 MAPK/extracellular-signal regulated kinase (ERK). Eur. J. Biochem. 268, 6058-6065.

Zhang J.Z., Behrooz A. and Ismail-Beigi F. (1999). Regulation of glucose transport by hypoxia. Am. J. Kidney Dis. 34, 189-202.

Zuscik M.J., Gunter T.E., Puzas J.E. and Rosier R.N. (1997). Characterization of voltage-sensitive calcium channels in growth plate chondrocytes. Biochem. Biophys. Res. Commun. 234, 432-438.

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