Glucosamine and osteoarthritis

Alexander R Shikhman
Scripps Clinic MS113, Division of Rheumatology, 10666 North Torey Pines Road, La Jolla, California 92037, USA
Tel.: +1 858 554 8562; Fax: +1 858 554 6763; ashikhman@scrippsclinic.com

Osteoarthritis is the most common form of arthritis and is commonly associated with significant disability. Glucosamine is widely used by patients with osteoarthritis as a nutriceutical in the USA and as a pharmaceutical in Europe. The efficacy of oral glucosamine, with regard to both symptom and disease modification in patients with osteoarthritis, remains controversial. This review analyzes the biological activities of glucosamine *in vivo* and *in vitro* and summarizes published data on clinical applications of this aminosugar in osteoarthritis.

Keywords: aminosugars, cartilage, chondrocyte, glucosamine, glucose transport, glycosaminoglycans, inflammation, osteoarthritis, pharmacokinetics, synovitis

future medicine

Osteoarthritis (OA) is the most common form of arthritis and is often associated with significant disability [1–3]. The impact of OA is expected to grow as the population increases and ages over the coming decades. The etiology of OA is multifactorial. OA-affected joints are characterized by progressive degeneration of the articular cartilage, osteophytes, subchondral sclerosis and alterations of the synovial membrane and joint capsule [4]. OA is a heterogeneous condition with differences in specific joint sites; these include apparent etiologies, as well as specific clinical, pathological or radiological features [4,5]. Typical clinical symptoms are pain and stiffness, particularly after prolonged activity [5,6]. Pathological processes in OA eventually result in joint deformities and significant functional impairment [4–6].

None of the therapies currently available are capable of delaying disease progression in OA [7]. Most treatment protocols are focused on pain and stiffness relief, and on the maintenance and improvement of functional status [7–9]. Treatment strategies for OA utilize both nonpharmacological and pharmacological modalities [7–9]. Nonpharmacological therapies include weight reduction, physiotherapy and orthotics. Pharmacological agents in OA are limited to [7–9]:

- Nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase (COX)-2 inhibitor(s)
- Simple analgesics (e.g., acetaminophen)
- Intra-articular therapy with corticosteroids and viscosupplementation devices
- Topical analgesics (e.g., capsaicin and NSAIDs)
- Over-the-counter supplements (e.g., glucosamine [GlcN] salts and chondroitin sulfate)

GlcN is commonly used by OA patients as a nutraceutical in the USA and as a pharmaceutical in Europe. However, the efficacy of oral GlcN, with regard to both symptom and disease modification in patients with knee OA, remains controversial [10]. This review analyzes the biological activities of GlcN *in vivo* and *in vitro* and summarizes published data on clinical applications of this aminosugar in OA.

Biological activities of glucosamine *in vitro*

GlcN expresses certain biological activities *in vitro* that may potentially contribute to its activity *in vivo*. These activities include:

- Interference with glucose transport and metabolism
- Modification of glycosaminoglycan synthesis
- Immunomodulation
- Inhibition of catabolic and proinflammatory responses
- Interference with chondrocyte survival

Glucosamine & glucose metabolism

Articular cartilage is an avascular tissue that receives its nutrients and oxygen by diffusion from blood vessels in the underlying bone and synovial fluid [11]. On a cellular basis, the consumption of oxygen by cartilage is only 2–5% of that of the liver or kidney. However, the amounts of lactate formed are comparable with those tissues [11], indicating that the energy generation in cartilage depends strongly on glucose supply [12]. Increasing concentrations of glucose suppress oxygen consumption (the Crabtree effect or catabolic repression), a phenomenon that has also been observed in articular chondrocytes [13]. Glucose is the main precursor for uridine-5´-phosphate (UDP)-hexosamines

and UDP-uronic acids, which are utilized by chondrocytes in glycosaminoglycan synthesis [14,15]. Therefore, changes in glucose uptake and metabolism affect quantitative and qualitative composition of cartilage matrix proteoglycans. Glucose concentration also modifies chondrocyte responses to certain growth factors, such as insulin-like growth factor (IGF)-1 [16], suggesting that glucose plays a key role in chondrocyte activation.

Transmembranous transport of glucose is facilitated by a group of highly specialized glucose-transporter proteins (GLUTs) [17]. Glucose transport in human articular chondrocytes is facilitated by at least five distinct GLUTs: cytokine-inducible GLUT-1 and -6 (formerly GLUT-9) and constitutively expressed GLUT-3, -8 and -10 [18,19]. Facilitated glucose transport in chondrocytes is insulin independent and regulated by proinflammatory cytokines and growth factors [18,19].

It has been well documented that in insulin-sensitive cells, such as myocytes and adipocytes, GlcN suppresses facilitated glucose transport and induces insulin resistance via the inhibition of the plasma membrane translocation of the insulin-dependent glucose transporter GLUT-4 [20]. Moreover, parenteral administration of GlcN also resulted in insulin resistance and suppressed GLUT-4 translocation to the cell membrane in skeletal muscle cells. Control infusions of galactosamine and mannosamine had no effect on glucose uptake and GLUT-4 membrane translocation [21]. The exact molecular pathways sustaining GlcN-induced insulin resistance are not completely understood. Among the current hypotheses explaining this phenomenon are: activation of protein kinase C and increased flux of glucose through the hexosamine pathway [22], depletion of ATP stores [23] and aberrant activation of Akt kinase [24].

GlcN inhibits glucose transport in unstimulated, cytokine- and growth-factor-stimulated human articular chondrocytes [25]. GlcN-regulated inhibition of glucose transport in chondrocytes is mediated via the suppression of expression and plasma membrane incorporation of inducible GLUT-1 and -6 proteins [25]. Owing to dependence of chondrocyte homeostasis on appropriate glucose supply, GlcN-induced changes in glucose transport can have profound effects on various chondrocyte functions.

Similar to glucose, imported GlcN is phosphorylated to GlcN-6-phosphate (P) by hexokinase in mammalian cells [26,27]. Accumulated GlcN-6-P inhibits hexokinase [27], which serves as a sensor and regulator of glucose transport [28–30].

The destiny of intracellular GlcN-6-P depends on the activity of GlcN-6-P deaminase/isomerase, the enzyme converting GlcN-6-P to fructose-6-P [31] that can be further utilized by glycolytic enzymes [32]. The expression and activity of GlcN-6-P deaminase in cartilage tissue and chondrocytes, as well as its ability to utilize supraphysiologic concentrations of intracellular GlcN-6-P, are unknown.

In contrast with glucose-6-P, GlcN-6-P demonstrates distinct activities toward glucose-utilizing metabolic pathways, including the hexosamine pathway, glycolysis and the pentose-monophosphate shunt.

GlcN-6-P enters the hexosamine pathway efficiently by bypassing glutamine–fructose 6-P amidotransferase, a gate-keeping enzyme of the hexosamine pathway [33], and via a series of nonrate-limiting enzymatic reactions is converted to UDP-*N*-acetylhexosamine [34,35] that can be utilized for glycosaminoglycan synthesis [36] and protein *O*-glycosylation [37]. However, GlcN-induced inhibition of glucose transport and GlcN-6-P allosteric inhibition of glutamine–fructose 6-P amidotransferase [38] reduces glucose flux through the hexosamine pathway. Therefore, the global effect of GlcN on glycosaminoglycan synthesis depends on the balance between these two processes.

GlcN and GlcN-6-P have a biphasic effect on glycolysis [39]. At low concentrations, they stimulate glycolysis and ATP production [39]. This may be explained, in part, by the conversion of GlcN-6-P to fructose-6-P, a substrate for glycolysis [31,32]. At a high concentration, GlcN and GlcN-6-P reduce lactate production and ATP accumulation [40] by inhibiting two enzymes of the glycolytic pathway: glyceraldehydes-3-P-dehydrogenase and lactate dehydrogenase [39].

The pentose-P shunt represents a vital metabolic pathway regulating energy generation and inactivation of reactive oxygen radicals [40,41]. It has been demonstrated that GlcN-6-P inhibits activity of glucose-6-P dehydrogenase (a key enzyme of the pentose-monophosphate shunt) in a dose-dependent fashion and suppresses nicotinamide adenine dinucleotide phosphate $(NADP)^+$ accumulation [42].

In summary, GlcN interferes with glucose transport and glucose metabolism in mammalian cells. Most of the measurable effects of

GlcN on glucose metabolism can only be achieved at GlcN concentrations exceeding the therapeutic concentrations by 10^2–10^3-times. Similar to other biological effects of GlcN, the observed *in vitro* interference of GlcN with glucose metabolism cannot be extrapolated directly onto its *in vivo* mode of action.

Glucosamine & cartilage glycosaminoglycan synthesis

Cartilage glycosaminoglycans are the key structural components of the extracellular cartilage matrix. Glycosaminoglycan loss is a feature of the aged and degenerated cartilage [43]. Prevention of glycosaminoglycan loss and acceleration of their synthesis are important components of chondroprotective strategies.

Different experimental approaches have been used to determine the effect of GlcN on extracellular cartilage matrix synthesis. GlcN can be imported by cells and incorporated into newly synthesized glycosaminoglycans [44]. UDP-*N*-acetylhexosamines, the end products of the hexosamine pathway, are the precursors for glycos-aminoglycan synthesis. Sweeney and colleagues demonstrated that, in Swarm rat chondrosarcoma cells, only one in 375 UDP-*N*-acetylhexosamines is derived from the exogenous GlcN in the presence of glucose [45]. By contrast, Noyszewski and colleagues demonstrated that GlcN is preferentially incorporated into galactosamine moieties of chondroitin sulfate at levels that were 300% higher than the equivalent amount of glucose [46]. Bassleer and colleagues reported that GlcN sulfate, at low and medium micromolar concentrations, accelerated the production of proteoglycans in cultured human articular chondrocytes without affecting the length of glycosaminoglycan chains and the rate of DNA and collagen II synthesis [47]. Mroz and Silbert showed that, at submillimolar concentrations, GlcN has no effect on sulphated glycosaminoglycan synthesis, whereas high concentrations of GlcN inhibit this process [48,49]. Similar findings were published by de Mattei and colleagues [50].

These variations among the experimental results, probably reflect the fact that GlcN plays a dual role in cell physiology: it efficiently enters the hexosamine pathway by bypassing glutamine–fructose 6-P amidotransferase, a gate-keeping enzyme of the hexosamine pathway, and enters the pool of UDP-*N*-acetylhexosamines [33–35]. However, it also inhibits facilitated glucose transport [25] and thus, can potentially decrease glucose flux through the hexosamine pathway. Therefore, the global effect of GlcN on glycosaminoglycan synthesis depends on the balance between these two pathways.

Glucosamine & chondrocyte activation by catabolic stimuli

It is well documented that proinflammatory cytokines, including interleukin (IL)-1β and tumor necrosis factor (TNF)-α, play a pathophysiological role in the development and progression of OA by initiating a sequence of catabolic events leading to the degradation of extracellular cartilage matrix and cartilage degeneration [51]. Treatment of articular chondrocytes with proinflammatory cytokines stimulates the production of collagen II degrading metalloproteinases [52], activates the matrix-degrading enzymes aggrecanase [53] and hexosaminidase [54], induces nitric oxide [55], COX-2 [56] and IL-6 [56] synthesis and suppresses the synthesis of sulfated glycosaminoglycans [57]. The increased production of IL-1β has been described in aged and OA cartilage [58]. Therefore, therapeutic agents possessing anticatabolic properties may potentially benefit chondroprotection.

Most of the existing data indicate that, at low millimolar concentrations, GlcN expresses anticatabolic and anti-inflammatory activities.

Pretreatment of equine cartilage explants with GlcN suppressed IL-1 and lipopolysaccharide-induced extracellular matrix degradation, as measured by a reduction of sulphated glycosaminoglycan release [59]. GlcN also inhibited IL-1- and lipopolysaccharide-induced nitric oxide and metalloproteinase release [59]. Previously, the author demonstrated that GlcN inhibited activation of human articular chondrocytes by IL-1β, as measured by nitric oxide production and expression of inducible nitric oxide synthase, COX-2 and IL-6 [60]. The inhibitory effect of GlcN on IL-1 responses did not depend on the suppression of IL-1-inducible mitogen-activated protein kinase (MAPK) activation or nuclear factor (NF)-κB nuclear translocation [60]. GlcN and several other hexosamines inhibited IL-1- and retinoic-acid-induced degradation of the extracellular matrix in rat chondrosarcoma cells and bovine cartilage explants via the inhibition of aggrecanase activity [61,62]. GlcN also inhibited the expression of another IL-1-inducible catabolic enzyme, stromelysin-1 [63]. Activation of chondrocytes

with IL-1 results in the suppression of sulphated glycosaminoglycan synthesis, which is mediated, in part, via the downregulation of glucuronosyltransferase-1 expression [63]. This inhibitory effect of IL-1 was prevented by GlcN [63].

At low millimolar concentrations, GlcN exhibited anticatabolic activity and delayed aggrecan degradation in bovine cartilage explants exposed to retinoic acid [64]. Byron and colleagues showed that, in cultured equine chondrocytes, GlcN inhibited mRNA and protein expression of several metalloproteinases, including matrix metalloproteinase (MMP)-1, -3 and -13, but had no effect on MMP activation [65]. Similarly, Dodge and Jimenez demonstrated the inhibitory effect of GlcN on MMP-3 production in human articular chondrocytes [66].

The exact molecular mechanisms mediating anticatabolic activity of GlcN are still poorly understood. The proposed mechanisms include: upregulation of IL-1β Type II decoy receptor [67], activation of protein kinase C [68], suppression of NF-κB nuclear translocation [67] and the inhibition of glycosylphosphatidylinositol-linked protein synthesis [69].

Thus, most of the published data indicate that GlcN expresses anticatabolic activity in cultured chondrocytes. The exact molecular mechanisms of GlcN-mediated anticatabolic activities remain to be defined. A major limitation of *in vitro* studies on GlcN is that its effects are observed predominantly at lower millimolar concentrations, which exceed the range of therapeutic concentrations obtained with oral GlcN by a factor of 10^2–10^3 [70–72].

Effect of glucosamine on immune & inflammatory responses

Inflammatory changes associated with OA include synovitis [73,74] and joint effusions [75]. They represent an important mechanism of joint pain in OA patients [75] and can contribute to the progression of cartilage degeneration [76]. Morphologically, OA synovium is characterized by synovial hyperplasia [74] and increased cellular infiltrates consisting of macrophages and $CD4^+$ helper T lymphocytes [77].

GlcN-mediated immunomodulation can also be a part of its anti-arthritic mechanisms. Ma and colleagues demonstrated that GlcN inhibited the induction and effector function of alloreactive cytotoxic murine T cells, suppressed activation of T lymphoblasts and dendritic cells, as well as allogeneic mixed leukocyte reactivity in a dose-dependent manner [78]. Furthermore, GlcN administration prolonged allogeneic cardiac allograft survival *in vivo* [78]. Similarly, Forchhammer and colleagues reported that, at low millimolar concentrations, GlcN suppressed unprimed T-cell responses by interfering with antigen-presenting cell functions and by a direct inhibitory effect on T-cell proliferation [79]. In addition, GlcN inhibited the secretion of cytokines in antigen-stimulated unprimed T cells and primed T-helper (Th)-2-polarized cells [79]. Yagita and colleagues reported that GlcN inhibited human natural killer cell activity [80]. By contrast, Matheson and colleagues described natural killer activity in normal human peripheral blood mononuclear cells as significantly elevated in the presence of 10^{-4} and 5×10^{-4} M GlcN [81].

GlcN is known also to modify neutrophil functions. GlcN suppresses superoxide anion generation and inhibits phagocytosis, the release of lysozyme and neutrophil chemotaxis [82]. In addition, GlcN reduces formyl methionyl leucylphenylalanine-induced upregulation of CD11b, polymerization of actin and phosphorylation of p38 MAPK [82].

Therefore, the anti-inflammatory property of GlcN can potentially contribute to the reduction of synovitis and symptom modification in arthritis. However, dose responses are still the main obstacles precluding the direct extrapolation of the *in vitro* data into *in vivo* models and clinical practice.

Glucosamine & apoptosis

Programmed cell death, or apoptosis, of chondrocytes is observed in cartilage impact injuries and may be part of the mechanisms leading to cartilage degeneration [83–85]. There are no data available indicating that GlcN protects chondrocytes from proapoptotic stimuli. On the contrary, supratherapeutic GlcN concentrations can induce cell death.

The ability of GlcN to induce cell death in malignant cells has been recognized for several decades. The cytostatic or cytotoxic effects of GlcN were reported for various tumor cell lines including L1210 leukemic cells [86], rat C6 glioma [87], mastocytoma P-815 [88], human colon carcinoma [89], hepatoma Mc-29 [90], myeloma protein in ascites (MOPC)-21 [91] and human malignant epithelial cells [92]. In most of the reports, the cytotoxic effect of GlcN was observed at low millimolar concentrations. Until recently, the cytotoxic effect of GlcN was considered as an almost exclusive attribute of transformed or malignant cells. However, in

2001, Nakamura and collaborators reported that GlcN induces apoptosis in cultured, non-transformed retinal neurons [93]. Later, de Mattei and colleagues showed that exposure of bovine cartilage explants to high concentrations of GlcN (above 20 mM) results in chondrocyte death [50].

The principal execution pathway (apoptosis vs oncosis) mediating GlcN-induced cell death is still undefined. Possible mechanisms of GlcN-induced cytotoxicity include:

- Surplus accumulation of UDP-*N*-acetylhexosamines [92]
- Interference with protein glycosylation [86]
- Inhibition of acetate incorporation into non-esterified sterols and lipids [94,95]
- Depletion of ATP stores [88]
- Inhibition of thymidine kinase [96]
- Inhibition of *N*-myristoyl transferase [97]

The author's data demonstrate that GlcN reproducibly induces cell death of human articular chondrocytes *in vitro* at concentrations exceeding 15 mM. GlcN-induced chondrocyte cell death represents a caspase-independent process. GlcN is also capable of inducing chondrocyte death upon its intra-articular administration [98].

Based on the dose–response curves, GlcN-induced chondrocyte death should not be a matter of concern in patients taking oral GlcN. However, it can represent a problem upon intra-articular administration of highly concentrated GlcN preparations.

Biological activities of glucosamine in animals with experimental arthritis

To determine whether GlcN possesses anti-arthritic activities *in vivo*, the author analyzed published articles regarding the *in vivo* effects of GlcN on inflammatory responses, cartilage morphology and cartilage biochemistry.

The effect of GlcN on synovitis was studied predominantly in small, experimental animals utilizing models of inflammatory arthropathies. The efficacy of GlcN in the therapy of synovitis associated with OA is a poorly studied topic.

Orally administered GlcN sulfate expressed anti-arthritic activity in rats with kaolin-induced arthritis and adjuvant arthritis [99]. The anti-inflammatory/anti-arthritic activity of GlcN was 50–300-fold lower than that of indomethacin [99]. GlcN expressed modest anti-inflammatory activity against edema induced by carrageenan, dextran and formalin. GlcN also protected rats against carrageenan-induced pleuritis. Furthermore, GlcN protected animals against peritonitis provoked in the rat by formalin and in the mouse by acetic acid [100]. GlcN did not show antinoceptive properties against pain provoked by intraperitoneal phenylquinone in mice. GlcN did not inhibit COX or proteolytic enzymes in the inflamed paw, but it was able to inhibit *in vitro* superoxide generation and lysosomal enzymes of the liver. The anti-inflammatory potency of GlcN was lower than that of acetylsalicylic acid, and much lower than that of indomethacin [100].

Oral administration of GlcN at supratherapeutic concentrations suppressed synovitis and cartilage degeneration induced by adjuvant arthritis in mice [101]. Similarly, oral administration of a combination of GlcN, chondroitin sulfate and manganese ascorbate in rats with collagen-induced arthritis significantly reduced arthritis severity [102].

Recently, the author demonstrated that intramuscular administration of GlcN in rabbits with experimental knee OA secondary to anterior cruciate ligament transection does not improve synovitis scores [103].

GlcN efficacy in experimental OA has mainly been studied in rabbits. Daily dietary supplementation with GlcN chloride at supratherapeutic concentrations did not significantly retard cartilage degeneration in rabbits with knee OA [104]. By contrast, daily administration of GlcN at therapeutic concentrations in rabbits with experimental knee OA induced by anterior cruciate ligament transection did not affect cartilage surface integrity (fibrillations and erosions) but improved focal safranin O scores in the lateral tibial plateaus. However, this was not confirmed by the biochemical analysis of sulfated glucosaminoglycan content [105]. Oegema and colleagues reported that oral administration of GlcN at supratherapeutic doses in rabbits with chymopapain-induced arthritis restored sulfated glycosaminoglycan content in the degenerated cartilage [106]. Oral GlcN did not affect sulfated glycosaminoglycan/proteoglycan content of the normal temporomandibular articular disc in rabbits [107]. Intramuscular administration of GlcN in rabbits with anterior cruciate ligament transection did not significantly affect OA scores in the treated animals, as compared with controls [103].

Kobayashi and colleagues analyzed the effect of GlcN (supratherapeutic doses) combined with chondroitin sulfate on cartilage degeneration in

rabbits after partial medial meniscectomy. The authors did not demonstrate any benefits of such a combination on cartilage scores, including MMP-1 scores, in the treated animals. However, addition of fursulthiamine, a vitamin B1 derivative, to the GlcN/chondroitin combination produced a measurable chondroprotective effect [108].

The analyzed published data did not reveal any benefits of GlcN administration on cartilage surface integrity (prevention of cartilage fibrillation and erosion) and on collagen synthesis and degradation.

In summary, most of the published data indicate that GlcN might express anti-inflammatory activity in animal models of inflammatory arthropathies. By contrast, the data on chondroprotective activity of GlcN in animals with experimental OA are still nonconclusive. Among the factors affecting the heterogeneity of these results are various routes and doses of the administered GlcN, as well as the absence of uniform criteria for the evaluation of GlcN chondroprotective activity in animals.

Glucosamine pharmacokinetics & toxicity

The pharmacokinetics of GlcN have been reviewed in detail in several publications [70,109]. Therefore, in this review, the author would like to emphasize several key points.

The absorption of orally administered GlcN is nearly 90% [70]. After absorption, GlcN is incorporated into plasma proteins during first-pass metabolism, resulting in 26% bioavailability [110]. A recent attempt to analyze the bioavailability of escalating doses of GlcN produced inconclusive results due to imperfections in the study design (absence of intravenous GlcN dosing, which is considered as 100% bioavailable) [111].

The baseline concentration of the endogenous serum GlcN in humans varies from 10 to 200 ng/ml [111]. After oral ingestion, the peak of serum GlcN occurs between 90 to 180 min, with a concentration range of 0.3–2.0 μg/ml [112].

Although GlcN exists mainly as an oral preparation, several topical formulations containing GlcN are also used in the therapy of OA [113]. Analysis of the transdermal permeability of GlcN in rats demonstrated a permeation rate of 13.27 $\mu g/cm^2/h$ at 5% concentration, which was considerably higher than the one predicted for hydrophilic substances [114].

Clinically relevant dosing of GlcN in humans and large animals (horses) results in serum and synovial fluid concentrations that are at least 500-fold lower than those reported to modify chondrocyte anabolic and catabolic activities in tissue- and cell-culture experiments [112,115].

Despite the fact that the currently recommended daily dose of oral GlcN is 1500 mg, formal analysis of the dose–response curves in OA symptom modification has never been performed in dedicated clinical trials.

Oral administration of GlcN at supratherapeutic doses (5000–15,000 mg/kg body weight) is well tolerated without documented toxicity. The lethal dose for 50% survival (LD_{50}) for GlcN for rats, mice and rabbits exceeds 5000 mg/kg, with a median value of more than 8000 mg/kg [109]. In small animals, GlcN has an excellent safety profile upon chronic and subchronic administration [116]. GlcN does not possess mutagenic activity [109]. Analysis of the clinical data demonstrates that GlcN does not possess toxicity upon chronic administration in humans [109].

It is known that administration of GlcN at supratherapeutic concentrations in animals induces insulin resistance [21,117]. Intravenous infusion of high-dose GlcN in humans also reproduced certain features of diabetes [118]. However, oral administration of GlcN in patients with OA has not been associated with an increased risk of glucose intolerance or diabetes [119,120].

Although available data indicate that chronic oral administration of GlcN at currently used doses (1500–3000 mg per day) does not possess a risk of major adverse events, systematic analysis of GlcN-associated cardiovascular or renal adverse events has never been performed. Furthermore, drug interactions involving GlcN is another practically unknown topic that requires further research.

Glucosamine use in patients with OA

GlcN was introduced to the therapy of OA almost 40 years ago [121,122]. The initial rationale for their use in OA was based on the notion that supplementation of patients with glycosaminoglycan precursors, including GlcN and its derivatives (*N*-acetylglucosamine, *N*-acetylgalactosamine, etc.) will accelerate glycosaminoglycan synthesis in articular cartilage and thus restore tissue integrity in the affected joints [121,122]. It has been well recognized that GlcN plays an important role in cartilage

homeostasis. Excessive urinary loss of this aminosugar, which takes place in patients with aspartylglucosaminuria, leads to the development of chronic arthritis [123]. The wide use of GlcN in OA was also stimulated by the description of its anticatabolic and anti-inflammatory properties *in vitro*. However, the main discrepancy between these *in vitro* experiments and the use of GlcN in humans is based on the fact that this aminosugar produces measurable biological effects *in vitro* when used at millimolar concentrations that exceed concentrations achievable in the joint upon oral administration by more than 100–1000-times [70–72]. This raises doubts that the effects observed *in vitro* represent a plausible mechanism of action *in vivo*.

Analysis of published reports indicates that most of the published clinical trials on GlcN are focused on symptom modification in patients with knee OA.

In 1980, Pujalte and colleagues reported that patients taking oral GlcN experienced a substantial reduction in joint pain as compared with those taking placebo [124]. Similar results were obtained in a multicenter clinical trial where 1200 patients with knee OA were treated with oral GlcN [125].

Intra-articular and intramuscular administration of GlcN was reported to reduce pain and increase the range of knee motion [126,127]. Using a different clinical protocol, D'Ambrosio and colleagues reported that, in patients with knee OA, parenteral (intravenous or intramuscular) administration of GlcN followed by oral intake diminished joint pain to a greater extent than placebo [128]. Oral GlcN produced analgesic effects similar to the NSAID drug ibuprofen [129,130]. By contrast with those studies, Houpt and colleagues noticed only marginal superiority of GlcN compared with placebo for pain relief in patients with knee OA [131]. Failure of GlcN to control knee pain in patients with OA was also reported by Rindone and coworkers [132]. Another double-blind, placebo-controlled trial summarized the outcomes of 212 patients with knee OA who were randomly treated with 1500 mg oral GlcN sulfate or placebo once daily for 3 years [133]. GlcN therapy significantly delayed joint space loss as compared with placebo. There were no significant differences in pain relief between the GlcN and the placebo group. In another randomized, placebo-controlled, double-blind trial of the relative effectiveness of GlcN sulfate and placebo in the managment of OA knee pain, analgesic activity of GlcN was not observed. However, those patients treated with GlcN demonstrated a statistically significant improvement in knee flexion [134]. Discontinuation of GlcN use in patients with knee OA, who previously benefited from GlcN administration, did not result in a statistically significant symptom exacerbation, indicating the absence of GlcN effect on OA symptoms [135].

Despite the marked consumption of GlcN by OA patients, a recently performed meticulous meta-analysis of the published clinical trials on GlcN in knee OA concluded that the therapeutic effect of GlcN on knee pain and function is still uncertain [10]. The meta-analysis included results of 24 published clinical trials on OA. The authors showed that, in the best designed studies, GlcN taken orally does not benefit knee pain and function [10]. Pooled results from the analyzed studies showed a trend towards superiority of GlcN sulfate versus GlcN chloride in the treatment of pain and functional impairment resulting from symptomatic OA [10]. However, no dedicated clinical trials have ever formally compared the efficacy of GlcN sulfate versus GlcN chloride in OA symptom modification. Hypothetically, one can propose that increased consumption of sulfate can potentially produce a mild cartilage-specific anabolic effect via interference with sulfation of the extracellular matrix glycosaminoglycans.

Recently, the National Institutes for Health summarized the data from a large multicentrer, placebo-controlled clinical trial comparing the symptom-modifying activity of GlcN chloride (alone and combined with chondroitin sulfate) with celecoxib and chondroitin sulfate in patients with symptomatic OA of the knee. Monotherapy with GlcN chloride did not reveal any superiority over placebo with regard to pain reduction in patients with both mild and moderate-to-severe knee pain. Combination therapy of GlcN chloride with chondroitin sulfate was found to be statistically superior to placebo only in patients with moderate-to-severe knee pain [136].

Several studies have attempted to analyze the effect of oral GlcN on cartilage metabolism utilizing surrogate biomarkers. Cibere and colleagues did not find statistically significant differences in urine and serum collagen type II fragment levels between patients with knee OA treated with GlcN sulfate versus placebo [137]. Similarly, Christgau and coworkers failed to demonstrate an effect of oral GlcN on urine collagen type II C telopeptide [138]. However,

patients with a high cartilage turnover were found to be more responsive to GlcN-mediated symptom modifications [138].

The efficacy of GlcN in patients with OA affecting joints other than the knee is practically unknown. Several published studies describe the results of the use of GlcN in combination with chondroitin sulfate to treat discogenic lower back [139] and OA affecting the temporomandibular joint [140]; however, the impact of GlcN when used in combination cannot be assessed accurately.

In summary, the existing dilemma of GlcN is based on the fact that its popularity among patients with OA is not strongly supported by the clinical evidence of its disease- or symptom-modifying activity.

Conclusions

Despite the currently existing controversies surrounding GlcN, this aminosugar remains a popular and widely consumed remedy to treat symptoms of OA. In academic settings, glucosamine represents a useful probe to study the molecular mechanisms of chondrocyte homeostasis and activation. Although GlcN expresses anti-inflammatory properties *in vivo*, its chondroprotective activity in animals with experimental OA is minimal, if any. The main obstacles preventing the universal acceptance of GlcN among physicians practicing evidence-based medicine are the lack of reproducible, unbiased and brand-independent data on the clinical utility of GlcN in OA and a marked gap between biologically efficient GlcN concentrations *in vitro* and therapeutically achievable GlcN concentrations *in vivo*.

Future perspective

The story of GlcN, obviously, is not over yet. Debates around GlcN re-emphasized the issue on the absence of current or foreseeable pharmaceutical agents possessing disease-modifying activity in OA and thus, are capable of preventing, or even restoring, cartilage integrity impaired by the disease. The lessons learnt from GlcN demonstrate that a simple monosaccharide can express a broad concentration-dependent range of biological effects *in vitro* that can be potentially exploited for the sake of chondroprotective interventions. Therefore, in the near future we can expect an emergence of GlcN derivatives or analogs combining chondroprotective and anti-inflammatory activities and having therapeutically attractive dose–response profiles both *in vitro* and *in vivo*. The discrepancy between the biologically efficient and therapeutically achievable GlcN concentrations will also be conquered by the introduction of GlcN analogs or derivatives suitable for transdermal or intra-articular applications.

Executive summary

Introduction

- Osteoarthritis (OA) is the most common form of arthritis characterized by the progressive degeneration of articular cartilage, osteophytes, subchondral sclerosis and alterations of the synovial membrane and joint capsule. None of the currently available therapies are capable of delaying disease progression in OA.
- The aminosugar glucosamine (GlcN) is commonly used by OA patients as a nutriceutical in the USA and as a pharmaceutical in Europe.

***Biological activities of glucosamine* in vitro**

- GlcN expresses certain biological activities *in vitro* that may potentially contribute to its activity *in vivo*. These activities include: 1) interference with glucose transport and metabolism; 2) modification of glycosaminoglycan synthesis; 3) immunomodulation; 4) inhibition of catabolic and proinflammatory responses; and 5) interference with chondrocyte survival.

Biological activities of glucosamine in animals with experimental arthritis

- GlcN possesses certain anti-inflammatory activities in animal models of inflammatory arthropathies. In contrast, the data on chondroprotective activity of GlcN in animals with experimental OA are still inconclusive.

Glucosamine use in patients with OA

- The clinical data on symptom- and disease-modifying activities of GlcN in OA are still controversial.

Conclusions

- The main discrepancy between *in vitro* GlcN activities and the use of this aminosugar in humans is based on the fact that GlcN produces measurable biological effects *in vitro* when used at millimolar concentrations. This exceeds the concentrations achievable in biological fluids upon oral administration by more than 100–1000-times.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Badley EM: The impact of disabling arthritis. *Arthritis Care Res.* 8(4), 221–228 (1995).
2. Morales-Torres J, Reginster JY, Hochberg MC: Rheumatic and musculoskeletal diseases and impaired quality of life: a challenge for rheumatologists. *J. Rheumatol.* 23(1), 1–3 (1996).
3. D'Ambrosia RD: Epidemiology of osteoarthritis. *Orthopedics* 28(2 Suppl.), S201–S205 (2005).
4. Buckwalter JA, Mankin HJ, Grodzinsky AJ: Articular cartilage and osteoarthritis. *Instr. Course Lect.* 54, 465–480 (2005).
5. Mandelbaum B, Waddell D: Etiology and pathophysiology of osteoarthritis. *Orthopedics* 28(2 Suppl.), S207–S214 (2005).
6. Dieppe PA, Lohmander LS: Pathogenesis and management of pain in osteoarthritis. *Lancet* 365(9463), 965–973 (2005).
7. Fajardo M, Di Cesare PE: Disease-modifying therapies for osteoarthritis: current status. *Drugs Aging* 22(2), 141–161 (2005).
8. Snibbe JC, Gambardella RA: Treatment options for osteoarthritis. *Orthopedics* 28(2 Suppl.), S215–S220 (2005).
9. Sarzi-Puttini P, Cimmino MA, Scarpa R *et al.*: Osteoarthritis: an overview of the disease and its treatment strategies. *Semin. Arthritis Rheum.* 35(1 Suppl.), 1–10 (2005).
10. Towheed TE, Maxwell L, Anastassiades TP *et al.*: Glucosamine therapy for treating osteoarthritis. *Cochrane Database Syst. Rev.* 18(2), CD002946 (2005).

• Important summary of clinical trials on glucosamine (GlcN) in osteoarthritis (OA).

11. Stockwell RA: Metabolism of cartilage. In: *Cartilage, Volume 1, Structure, Function and Biochemistry*. Hall BK (Ed.). Academic Press, NY, USA, 253–280 (1983).
12. Rajpurohit R, Koch CJ, Tao Z *et al.*: Adaptation of chondrocytes to low oxygen tension: relationship between hypoxia and cellular metabolism. *J. Cell Physiol.* 168(2), 424–432 (1996).
13. Otte P: Basic cell metabolism of articular cartilage. Manometric studies. *Z. Rheumatol.* 50(5), 304–312 (1991).
14. Kim JJ, Conrad HE: Kinetics of mucopolysaccharide and glycoprotein synthesis by chick embryo chondrocytes. Effect of D-glucose concentration in the culture medium. *J. Biol. Chem.* 251(20), 6210–6217 (1976).
15. Mason RM, Sweeney C: The relationship between proteoglycan synthesis in swarm chondrocytes and pathways of cellular energy and UDP-sugar metabolism. *Carbohydr. Res.* 255, 255–270 (1994).
16. Kelley KM, Johnson TR, Ilan J, Moskowitz RW: Glucose regulation of the IGF response system in chondrocytes: induction of an IGF-I-resistant state. *Am. J. Physiol.* 276(4 Pt 2), R1164–R1171 (1999).
17. Uldry M, Thorens B: The SLC2 family of facilitated hexose and polyol transporters. *Pflugers Arch.* 447(5), 480–489 (2004).
18. Shikhman AR, Brinson DC, Valbracht J, Lotz MK: Cytokine regulation of facilitated glucose transport in human articular chondrocytes. *J. Immunol.* 167(12), 7001–7008 (2001).
19. Shikhman AR, Brinson DC, Lotz MK: Distinct pathways regulate facilitated glucose transport in human articular chondrocytes during anabolic and catabolic responses. *Am. J. Physiol. Endocrinol. Metabol.* 286(6), E980–E985 (2004).
20. Chen H, Ing BL, Robinson KA *et al.*: Effects of overexpression of glutamine: fructose-6-phosphate amidotransferase (GFAT) and glucosamine treatment on translocation of GLUT4 in rat adipose cells *Mol. Cell Endocrinol.* 135(1), 67–77 (1997).
21. Baron AD, Zhu JS, Zhu JH *et al.*: Glucosamine induces insulin resistance *in vivo* by affecting GLUT 4 translocation in skeletal muscle. Implications for glucose toxicity. *J. Clin. Invest.* 96(6), 2792–2801 (1995).
22. Filippis A, Clark S, Proietto J: Increased flux through the hexosamine biosynthesis pathway inhibits glucose transport acutely by activation of protein kinase C. *Biochem. J.* 324(Pt 3), 981–985 (1997).
23. Hresko RC, Heimberg H, Chi MM, Mueckler M: Glucosamine-induced insulin resistance in 3T3-L1 adipocytes is caused by depletion of intracellular ATP. *J. Biol. Chem.* 273(32), 20658–20668 (1998).
24. Heart E, Choi WS, Sung CK: Glucosamine-induced insulin resistance in 3T3-L1 adipocytes. *Am. J. Physiol. Endocrinol. Metabol.* 278(1), E103–E112 (2000).
25. Shikhman AR, Brinson DC, Lotz MK: Facilitated transport of glucose, glucosamine and *N*-acetylglucosamine in human articular chondrocytes. *Osteoarthritis Cartilage* 11(Suppl. A), S93 (2003).
26. Marshall S, Yamasaki K, Okuyama R: Glucosamine induces rapid desensitization of glucose transport in isolated adipocytes by increasing GlcN-6-P levels. *Biochem. Biophys. Res. Commun.* 329(3), 1155–1161 (2005).
27. Virkamaki A, Yki-Jarvinen H: Allosteric regulation of glycogen synthase and hexokinase by glucosamine-6-phosphate during glucosamine-induced insulin resistance in skeletal muscle and heart. *Diabetes* 48(5), 1101–1107 (1999).
28. Postic C, Shiota M, Magnuson MA: Cell-specific roles of glucokinase in glucose homeostasis. *Recent Prog. Horm. Res.* 56, 195–217 (2001).
29. Nagamatsu S, Nakamichi Y, Sawa H: Lucose transporter expression and functional role of hexokinase in insulin biosynthesis in mouse β TC3 cells. *Am. J. Physiol.* 269(2 Pt 1), C480–C486 (1995).
30. Allen CB, Guo XL, White CW: Changes in pulmonary expression of hexokinase and glucose transporter mRNAs in rats adapted to hyperoxia. *Am. J. Physiol.* 274(3 Pt 1), L320–L329 (1998).
31. Shevchenko V, Hogben M, Ekong R *et al.*: The human glucosamine-6-phosphate deaminase gene: cDNA cloning and expression, genomic organization and chromosomal localization. *Gene* 216(1), 31–38 (1998).
32. Malaisse WJ, Zhang Y, Sener A: Enzyme-to-enzyme channeling in the early steps of glycolysis in rat pancreatic islets. *Endocrine* 24(2), 105–109 (2004).
33. Heart E, Choi WS, Sung CK: Glucosamine-induced insulin resistance in 3T3-L1 adipocytes. *Am. J. Physiol. Endocrinol. Metabol.* 278(1), E103–E112 (2000).
34. Robinson KA, Sens DA, Buse MG: Pre-exposure to glucosamine induces insulin resistance of glucose transport and glycogen synthesis in isolated rat skeletal muscles. Study of mechanisms in muscle and in rat-1 fibroblasts overexpressing the human insulin receptor. *Diabetes* 42(9), 1333–1346 (1993).
35. Kornfeld R: Studies on L-glutamine D-fructose 6-phosphate amidotransferase. I. Feedback inhibition by uridine diphosphate-*N*-acetylglucosamine. *J. Biol. Chem.* 242(13), 3135–3141 (1967).
36. Prydz K, Dalen KT: Synthesis and sorting of proteoglycans. *J. Cell Sci.* 113(Pt 2), 193–205 (2000).
37. Wells L, Hart GW: O-GlcNAc turns twenty: functional implications for post-translational modification of nuclear and cytosolic proteins with a sugar. *FEBS Lett.* 546(1), 154–158 (2003).
38. Broschat KO, Gorka C, Page JD *et al.*: Kinetic characterization of human glutamine-fructose-6-phosphate amidotransferase I: potent feedback inhibition by glucosamine 6-phosphate. *J. Biol. Chem.* 277(17), 14764–14770 (2002).

39. Tesoriere G, Vento R, Calvaruso G: Inhibitory effect of D-glucosamine on glycolysis in bovine retina. *Biochim. Biophys. Acta* 385(1), 58–67 (1975).
40. Gerbitz KD, Gempel K, Brdiczka D: Mitochondria and diabetes genetic, biochemical, and clinical implications of the cellular energy circuit. *Diabetes* 45(2), 113–126 (1996).
41. Kletzien RF, Harris PK, Foellmi LA: Glucose-6-phosphate dehydrogenase: a 'housekeeping' enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. *FASEB J.* 8(2), 174–181 (1994).
42. Wang XT, Au SW, Lam VM, Engel PC: Recombinant human glucose-6-phosphate dehydrogenase. Evidence for a rapid-equilibrium random-order mechanism. *Eur. J. Biochem.* 269(14), 3417–3424 (2002).
43. Caterson B, Flannery CR, Hughes CE, Little CB: Mechanisms involved in cartilage proteoglycan catabolism. *Matrix Biol.* 19(4), 333–344 (2000).
44. Kleine TO, Stephan R: Biosynthesis of chondroitin sulfate side chains and hyaluronate. Time-course studies with cartilage slices of calf ribs under different conditions. *Biochim. Biophys. Acta* 451(2), 444–456. (1976).
45. Sweeney C, Mackintosh D, Mason RM: UDP-sugar metabolism in Swarm rat chondrosarcoma chondrocytes. *Biochem. J.* 290(Pt 2), 563–570 (1993).
46. Noyszewski EA, Wroblewski K, Dodge GR *et al.*: Preferential incorporation of glucosamine into the galactosamine moieties of chondroitin sulfates in articular cartilage explants. *Arthritis Rheum.* 44(5), 1089–1095 (2001).
47. Bassleer C, Rovati L, Franchimont P: Stimulation of proteoglycan production by glucosamine sulfate in chondrocytes isolated from human osteoarthritic articular cartilage *in vitro*. *Osteoarthritis Cartilage* 6(6), 427–434 (1998).
48. Mroz PJ, Silbert JE: Effects of (3H)glucosamine concentration on (3H)chondroitin sulfate formation by cultured chondrocytes. *Biochem. J.* 376(Pt 2), 511–515 (2003).
49. Mroz PJ, Silbert JE: Use of 3H-glucosamine and 35S-sulfate with cultured human chondrocytes to determine the effect of glucosamine concentration on formation of chondroitin sulfate. *Arthritis Rheum.* 50(11), 3574–3579 (2004).
50. de Mattei M, Pellati A, Pasello M *et al.*: High doses of glucosamine-HCl have detrimental effects on bovine articular cartilage explants cultured *in vitro*. *Osteoarthritis Cartilage* 10(10), 816–825 (2002).
51. Goldring SR, Goldring MB: The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clin. Orthop.* 427(Suppl.), S27–S36 (2004).
52. Vincenti MP, Brinckerhoff CE: Transcriptional regulation of collagenase (*MMP-1*, *MMP-13*) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res.* 4(3), 157–164 (2002).
53. Pratta MA, Scherle PA, Yang G *et al.*: Induction of aggrecanase 1 (ADAM-TS4) by interleukin-1 occurs through activation of constitutively produced protein. *Arthritis Rheum.* 48(1), 119–133 (2003).
54. Shikhman AR, Brinson DC, Lotz M: Profile of glycosaminoglycan-degrading glycosidases and glycoside sulfatases secreted by human articular chondrocytes in homeostasis and inflammation. *Arthritis Rheum.* 43(6), 1307–1314 (2000).
55. Abramson SB, Attur M, Amin AR, Clancy R: Nitric oxide and inflammatory mediators in the perpetuation of osteoarthritis. *Curr. Rheumatol. Rep.* 3(6), 535–541 (2001).
56. Lotz M: Cytokines in cartilage injury and repair. *Clin. Orthop.* 391(Suppl.), S108–S115 (2001).
57. Gouze JN, Bordji K, Gulberti S *et al.*: Interleukin-1β downregulates the expression of glucuronosyltransferase I, a key enzyme priming glycosaminoglycan biosynthesis: influence of glucosamine on interleukin-1β-mediated effects in rat chondrocytes. *Arthritis Rheum.* 44(2), 351–360 (2001).
58. Loeser RF, Carlson CS, Del Carlo M, Cole A: Detection of nitrotyrosine in aging and osteoarthritic cartilage: Correlation of oxidative damage with the presence of interleukin-1β and with chondrocyte resistance to insulin-like growth factor 1. *Arthritis Rheum.* 46(9), 2349–2357 (2002).
59. Fenton JI, Chlebek-Brown KA, Peters TL *et al.*: Glucosamine HCl reduces equine articular cartilage degradation in explant culture. *Osteoarthritis Cartilage* 8(6), 258–265 (2000).
60. Shikhman AR, Kuhn K, Alaaeddine N, Lotz M: *N*-acetylglucosamine prevents IL-1 β-mediated activation of human chondrocytes. *J. Immunol.* 166(8), 5155–5160 (2001).
- Comparison of the anti-inflammatory activity of various aminosugars.
61. Sandy JD, Gamett D, Thompson V, Verscharen C: Chondrocyte-mediated catabolism of aggrecan: aggrecanase-dependent cleavage induced by interleukin-1 or retinoic acid can be inhibited by glucosamine. *Biochem. J.* 335(Pt 1), 59–66 (1998).
- Description of GlcN-mediated anti-inflammatory mechanisms.
62. Patwari P, Kurz B, Sandy JD, Grodzinsky AJ: Mannosamine inhibits aggrecanase-mediated changes in the physical properties and biochemical composition of articular cartilage. *Arch. Biochem. Biophys.* 374(1), 79–85 (2000).
63. Gouze JN, Bordji K, Gulberti S *et al.*: Interleukin-1β downregulates the expression of glucuronosyltransferase I, a key enzyme priming glycosaminoglycan biosynthesis: influence of glucosamine on interleukin-1β-mediated effects in rat chondrocytes. *Arthritis Rheum.* 44(2), 351–360 (2001).
64. Ilic MZ, Martinac B, Handley CJ: Effects of long-term exposure to glucosamine and mannosamine on aggrecan degradation in articular cartilage. *Osteoarthritis Cartilage* 11(8), 613–622 (2003).
65. Byron CR, Orth MW, Venta PJ *et al.*: Influence of glucosamine on matrix metalloproteinase expression and activity in lipopolysaccharide-stimulated equine chondrocytes. *Am. J. Vet. Res.* 64(6), 666–671 (2003).
66. Dodge GR, Jimenez SA: Glucosamine sulfate modulates the levels of aggrecan and matrix metalloproteinase-3 synthesized by cultured human osteoarthritis articular chondrocytes. *Osteoarthritis Cartilage* 11(6), 424–432 (2003).
67. Gouze JN, Bianchi A, Becuwe P *et al.*: Glucosamine modulates IL-1-induced activation of rat chondrocytes at a receptor level, and by inhibiting the NF-κB pathway. *FEBS Lett.* 510(3), 166–170 (2002).
- Description of GlcN-mediated anti-inflammatory mechanisms.
68. Piperno M, Reboul P, Hellio Le Graverand MP *et al.*: Glucosamine sulfate modulates dysregulated activities of human osteoarthritic chondrocytes *in vitro*. *Osteoarthritis Cartilage* 8(3), 207–212 (2000).
69. Sandy JD, Thompson V, Verscharen C, Gamett D: Chondrocyte-mediated catabolism of aggrecan: evidence for a glycosylphosphatidylinositol-linked protein in the aggrecanase response to interleukin-1 or retinoic acid. *Arch. Biochem. Biophys.* 367(2), 258–264 (1999).

70. Setnikar I, Rovati LC: Absorption, distribution, metabolism and excretion of glucosamine sulfate. A review. *Arzneimittelforschung* 51(9), 699–725 (2001).
• Review of GlcN pharmacokinetics.
71. Adebowale A, Du J, Liang Z *et al.*: The bioavailability and pharmacokinetics of glucosamine hydrochloride and low molecular weight chondroitin sulfate after single and multiple doses to beagle dogs. *Biopharm. Drug Dispos.* 23(6), 217–225 (2002).
72. Aghazadeh-Habashi A, Sattari S, Pasutto F, Jamali F: Single dose pharmacokinetics and bioavailability of glucosamine in the rat. *J. Pharm. Sci.* 5(2), 181–184 (2002).
73. Rhodes LA, Conaghan PG, Radjenovic A *et al.*: Further evidence that a cartilage-pannus junction synovitis predilection is not a specific feature of rheumatoid arthritis. *Ann. Rheum. Dis.* 64(9), 1347–1349 (2005).
74. Fernandez-Madrid F, Karvonen RL, Teitge RA *et al.*: Synovial thickening detected by MR imaging in osteoarthritis of the knee confirmed by biopsy as synovitis. *Magn. Reson. Imaging* 13(2), 177–183 (1995).
75. Hill CL, Gale DG, Chaisson CE *et al.*: Knee effusions, popliteal cysts, and synovial thickening: association with knee pain in osteoarthritis. *J. Rheumatol.* 28(6), 1330–1337 (2001).
76. Ayral X, Pickering EH, Woodworth TG *et al.*: Synovitis: a potential predictive factor of structural progression of medial tibiofemoral knee osteoarthritis – results of a 1 year longitudinal arthroscopic study in 422 patients. *Osteoarthritis Cartilage* 13(5), 361–367 (2005).
77. Saito I, Koshino T, Nakashima K *et al.*: Increased cellular infiltrate in inflammatory synovia of osteoarthritic knees. *Osteoarthritis Cartilage* 10(2), 156–162 (2002).
78. Ma L, Rudert WA, Harnaha J *et al.*: Immunosuppressive effects of glucosamine. *J. Biol. Chem.* 277(42), 39343–39349 (2002).
79. Forchhammer L, Thorn M, Met O *et al.*: Immunobiological effects of glucosamine *in vitro*. *Scand. J. Immunol.* 58(4), 404–411 (2003).
80. Yagita M, Nakajima M, Saksela E: Suppression of human natural killer cell activity by amino sugars. *Cell Immunol.* 122(1), 83–95 (1989).
81. Matheson DS, Green BJ, Friedman SJ: Effect of D-glucosamine on human natural killer activity *in vitro*. *J. Biol. Response Mod.* 3(4), 445–453 (1984).
82. Hua J, Sakamoto K, Nagaoka I: Inhibitory actions of glucosamine, a therapeutic agent for osteoarthritis, on the functions of neutrophils. *J. Leukoc. Biol.* 71(4), 632–640 (2002).
83. D'Lima DD, Hashimoto S, Chen PC *et al.*: Impact of mechanical trauma on matrix and cells. *Clin. Orthop.* 391(Suppl.), S90–S99 (2001).
84. Borrelli J Jr, Tinsley K, Ricci WM *et al.*: Induction of chondrocyte apoptosis following impact load. *J. Orthop. Trauma* 17(9), 635–641 (2003).
85. Vrahas MS, Mithoefer K, Joseph D: The long-term effects of articular impaction. *Clin. Orthop.* 423, 40–43 (2004).
86. Morin MJ, Porter CW, McKernan P, Bernacki RJ: The biochemical and ultrastructural effects of tunicamycin and D-glucosamine in L1210 leukemic cells. *J. Cell Physiol.* 114(2), 162–172 (1983).
87. Calvaruso G, Taibi G, Torregrossa MV *et al.*: Uridine enhances the cytotoxic effect of D-glucosamine in rat C6 glioma cells. *Life Sci.* 39(23), 2221–2227 (1986).
88. Ichikawa A, Takagi M, Tomita K: Effect of D-glucosamine on growth and several functions of cultured mastocytoma P-815 cells. *J. Pharmacobiodyn.* 3(11), 577–588 (1980).
89. Krug E, Zweibaum A, Schulz-Holstege C, Keppler D: D-glucosamine-induced changes in nucleotide metabolism and growth of colon-carcinoma cells in culture. *Biochem. J.* 217(3), 701–708 (1984).
90. Chelibonova-Lorer H, Gavazova E, Ivanov S, Antonova M: Effect of D-glucosamine on the content and synthesis of UDP-sugars and plasma membrane associated carbohydrates in chicken liver and hepatoma Mc-29. *Int. J. Biochem.* 15(4), 559–564 (1983).
91. Chelibonova-Lorer H: Effect of D-glucosamine and D-galactosamine on the uridine nucleotide concentration in mouse myeloid tumor (Graffi) and myeloma MOPC-21. *Neoplasma* 22(1), 23–27 (1975).
92. Krug E, Dussaulx E, Rozen R *et al.*: D-glucosamine-induced increase of the glycerol-containing lipids in growing cultures of human malignant epithelial cells. *J. Natl Cancer Inst.* 70(1), 57–61 (1983).
93. Nakamura M, Barber AJ, Antonetti DA *et al.*: Excessive hexosamines block the neuroprotective effect of insulin and induce apoptosis in retinal neurons. *J. Biol. Chem.* 276(47), 43748–43755 (2001).
94. Bernacki RJ, Sharma M, Porter NK *et al.*: Biochemical characteristics, metabolism, and antitumor activity of several acetylated hexosamines. *J. Supramol. Struct.* 7(2), 235–250, (1977).
95. Friedman SJ, Cheng S, Skehan P: Mechanisms of cholesterol synthesis inhibition by D-glucosamine. *Can. J. Biochem. Cell Biol.* 63(12), 1253–1257 (1985).
96. Friedman SJ, Kimball T, Trotter CD, Skehan PJ: The inhibition of thymidine kinase in glial tumor cells by an amino sugar, D-glucosamine. *Cancer Res.* 37(4), 1068–1074 (1977).
97. Jacobsen C, Andreassen H, Saermark T: Inhibition by glucosamine of myristoylation in human H9 lymphocytes and rat liver cells. *FEBS Lett.* 259(1), 91–94 (1989).
98. Shikhman AR, Kuhn K, Zenmyo M, Lotz MK: Aminosugars and chondrocytes apoptosis. *Osteoarthritis Cartilage* 11(Suppl. A), S52 (2003).
99. Setnikar I, Pacini MA, Revel L: Anti-arthritic effects of glucosamine sulfate studied in animal models. *Arzneimittelforschung* 41(5), 542–545 (1991).
100. Setnikar I, Cereda R, Pacini MA, Revel L: Antireactive properties of glucosamine sulfate. *Arzneimittelforschung* 41(2), 157–161 (1991).
101. Hua J, Suguro S, Hirano S *et al.*: Preventive actions of a high dose of glucosamine on adjuvant arthritis in rats. *Inflamm. Res.* 54(3), 127–132 (2005).
102. Beren J, Hill SL, Diener-West M, Rose NR: Effect of pre-loading oral glucosamine HCl/chondroitin sulfate/manganese ascorbate combination on experimental arthritis in rats. *Exp. Biol. Med.* 226(2), 144–151 (2001).
103. Shikhman AR, Amiel D, D'Lima D *et al.*: Chondroprotective activity of *N*-acetylglucosamine in rabbits with experimental osteoarthritis. *Ann. Rheum. Dis.* 64(1), 89–94 (2005).
104. Lippiello L, Woodward J, Karpman R, Hammad TA: *In vivo* chondroprotection and metabolic synergy of glucosamine and chondroitin sulfate. *Clin. Orthop.* 381, 229–240 (2000).
105. Tiraloche G, Girard C, Chouinard L *et al.*: Effect of oral glucosamine on cartilage degradation in a rabbit model of osteoarthritis. *Arthritis Rheum.* 52(4), 1118–1128 (2005).
106. Oegema TR Jr, Deloria LB, Sandy JD, Hart DA: Effect of oral glucosamine on cartilage and meniscus in normal and chymopapain-injected knees of young rabbits. *Arthritis Rheum.* 46(9), 2495–2503 (2002).
107. Deniz M, Oegema TR Jr, Schiffman EL, Look JO: The effect of exogenous glucosamine hydrochloride on the proteoglycan concentration of the articular disc of the rabbit temporomandibular joint. *J. Orofac. Pain* 17(3), 251–253 (2003).

108. Kobayashi T, Notoya K, Nakamura A, Akimoto K: Fursultiamine, a vitamin B1 derivative, enhances chondroprotective effects of glucosamine hydrochloride and chondroitin sulfate in rabbit experimental osteoarthritis. *Inflamm. Res.* 54(6), 249–255 (2005).
109. Anderson JW, Nicolosi RJ, Borzelleca JF: Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. *Food Chem. Toxicol.* 43(2), 187–201 (2005).
• Review of GlcN pharmacokinetics and toxicity.
110. Barclay TS, Tsourounis C, McCart GM: Glucosamine. *Ann. Pharmacother.* 32(5), 574–579 (1998).
111. Persiani S, Roda E, Rovati LC *et al.*: Glucosamine oral bioavailability and plasma pharmacokinetics after increasing doses of crystalline glucosamine sulfate in man. *Osteoarthritis Cartilage* 13, 1041–1049 (2005).
112. Biggee BA, Blinn CM, McAlindon TE *et al.*: Low levels of human serum glucosamine after ingestion of glucosamine sulfate relative to capability for peripheral effectiveness. *Ann. Rheum. Dis.* [Epub ahead of print] (2005).
113. Cohen M, Wolfe R, Mai T, Lewis D: A randomized, double-blind, placebo-controlled trial of a topical cream containing glucosamine sulfate, chondroitin sulfate, and camphor for osteoarthritis of the knee. *J. Rheumatol.* 30(3), 523–528 (2003).
114. Kanwischer M, Kim SY, Kim JS *et al.*: Evaluation of the physicochemical stability and skin permeation of glucosamine sulfate. *Drug Dev. Ind. Pharm.* 31(1), 91–97 (2005).
115. Laverty S, Sandy JD, Celeste C *et al.*: Synovial fluid levels and serum pharmacokinetics in a large animal model following treatment with oral glucosamine at clinically relevant doses. *Arthritis Rheum.* 52(1), 181–191 (2005).
116. Echard BW, Talpur NA, Funk KA *et al.*: Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats. *Mol. Cell Biochem.* 225(1), 85–91 (2001).
117. Rossetti L, Hawkins M, Chen W *et al.*: *In vivo* glucosamine infusion induces insulin resistance in normoglycemic but not in hyperglycemic conscious rats. *J. Clin. Invest.* 96(1), 132–140 (1995).
118. Monauni T, Zenti MG, Cretti A *et al.*: Effects of glucosamine infusion on insulin secretion and insulin action in humans. *Diabetes* 49(6), 926–935 (2000).
119. Tannis AJ, Barban J, Conquer JA: Effect of glucosamine supplementation on fasting and non-fasting plasma glucose and serum insulin concentrations in healthy individuals. *Osteoarthritis Cartilage* 12(6), 506–511 (2004).
120. Scroggie DA, Albright A, Harris MD: The effect of glucosamine-chondroitin supplementation on glycosylated hemoglobin levels in patients with Type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. *Arch. Intern. Med.* 163(13), 1587–1590 (2003).
121. Vetter G: Glucosamine in the therapy of degenerative rheumatism. *Dtsch. Med. J.* 16(3), 446–449 (1965).
122. Vetter G: Topical therapy of arthroses with glucosamines (Dona200). *Munch. Med. Wochenschr.* 111(28), 1499–1502 (1969).
123. Arvio MA, Rapola JM, Pelkonen PM: Chronic arthritis in patients with aspartylglucosaminuria. *J. Rheumatol.* 25(6), 1131–1134 (1998).
124. Pujalte JM, Llavore EP, Ylescupidez FR: Double-blind clinical evaluation of oral glucosamine sulfate in the basic treatment of osteoarthrosis. *Curr. Med. Res. Opin.* 7(2), 110–114 (1980).
125. Tapadinhas MJ, Rivera IC, Bignamini AA: Oral glucosamine sulfate in the management of arthrosis: report on a multi-centre open investigation in Portugal. *Pharmatherapeutica* 3(3), 157–168 (1982).
126. Vajaradul Y: Double-blind clinical evaluation of intra-articular glucosamine in out-patients with gonarthrosis. *Clin. Ther.* 3(5), 336–343 (1981).
127. Reichelt A, Forster KK, Fischer M *et al.*: Efficacy and safety of intramuscular glucosamine sulfate in osteoarthritis of the knee. A randomised, placebo-controlled, double-blind study. *Arzneimittelforschung* 44(1), 75–80 (1994).
128. D'Ambrosio E, Casa B, Bompani R *et al.*: Glucosamine sulfate: a controlled clinical investigation in arthrosis. *Pharmatherapeutica* 2(8), 504–508 (1981).
129. Lopes Vaz A: Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulfate in the management of osteoarthrosis of the knee in out-patients. *Curr. Med. Res. Opin.* 8(3), 145–149 (1982).
130. Qiu GX, Gao SN, Giacovelli G *et al.*: Efficacy and safety of glucosamine sulfate versus ibuprofen in patients with knee osteoarthritis. *Arzneimittelforschung* 48, 469–474 (1998).
131. Houpt JB, McMillan R, Wein C, Paget-Dellio SD: Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. *J. Rheumatol.* 26(11), 2423–2430, (1999).
132. Rindone JP, Hiller D, Collacott E *et al.*: Randomized, controlled trial of glucosamine for treating osteoarthritis of the knee. *West. J. Med.* 172(2), 91–94 (2000).
133. Reginster JY, Deroisy R, Rovati LC *et al.*: Long-term effects of glucosamine sulfate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. *Lancet* 357(9252), 251–256 (2001).
• Frequently quoted clinical trial on GlcN in OA.
134. Hughes R, Carr A: A randomized, double-blind, placebo-controlled trial of glucosamine sulfate as an analgesic in osteoarthritis of the knee. *Rheumatology* 41(3), 279–284 (2002).
135. Cibere J, Kopec JA, Thorne A *et al.*: Randomized, double-blind, placebo-controlled glucosamine discontinuation trial in knee osteoarthritis. *Arthritis Rheum.* 51(5), 738–745 (2004).
136. Clegg DO, Reda DJ, Harris CL *et al.*: Efficacy of glucosamine and chondroitin sulfate in patients with painful knee osteoarthritis (OA): the glucosamine/chondroitin arthritis intervention trial (GAIT). *Arthritis Rheum.* 52(9 Suppl.) S256 (2005).
137. Cibere J, Thorne A, Kopec JA *et al.*: Glucosamine sulfate and cartilage Type II collagen degradation in patients with knee osteoarthritis: randomized discontinuation trial results employing biomarkers. *J. Rheumatol.* 32(5), 896–902 (2005).
138. Christgau S, Henrotin Y, Tanko LB *et al.*: Osteoarthritic patients with high cartilage turnover show increased responsiveness to the cartilage protecting effects of glucosamine sulfate. *Clin. Exp. Rheumatol.* 22(1), 36–42 (2004).
139. Klein RG, Eek BC, O'Neill CW *et al.*: Biochemical injection treatment for discogenic low back pain: a pilot study. *Spine J.* 3(3), 220–226 (2003).
140. Shankland WE 2nd: The effects of glucosamine and chondroitin sulfate on osteoarthritis of the TMJ: a preliminary report of 50 patients. *Cranio* 16(4), 230–235 (1998).

Affiliation

• *Alexander R Shikhman, MD, PhD*
Scripps Clinic MS113, Division of Rheumatology, 10666 North Torey Pines Road, La Jolla, California 92037, USA
Tel.: +1 858 554 8562;
Fax: +1 858 554 6763;
ashikhman@scrippsclinic.com