Relief First Capsules
Scientific White Paper

"A Prominent Ph.D., Reviews 266 Pieces Of Reliable & Credible Scientific Research And Prepares A 40 Page Document On The Ingredients In The Relief First Capsules!"

Keep reading this special report to find out about the scientific literature that was reviewed on articular cartilage biochemistry and physiology, cartilage degeneration, degenerative joint disease and osteoarthritis that led Michael John Glade, Ph.D., to conclude that the ingredients in...

Relief First capsules are a natural COX-2 inhibitor.

Relief First capsules do not have the health risks associated with prescription COX-2 Inhibitors.

Relief First capsules are good for people of any age.

Dear Concerned Consumer,

Joint health and everyday joint aches and minor pains affect most of us at some time in our lives. We all have relatives and friends who suffer discomfort in the back, neck and extremities. The goal for all of us is to achieve effective, but also safe, management of these musculoskeletal disorders.

That's why a prominent and highly regarded scientist, Michael John Glade, Ph.D., F.A.C.N., C.N.S., was asked to review the database of the National Library of Medicine and the National Institutes of Health Office of Dietary Supplements to determine the safety and efficacy of the ingredients in the Relief First capsules.

Dr. Glade's scientific literature search used the extensive "PubMed" electronic database that includes citations of over 10,000 scientific articles published in over 4,000 journals. In addition, Dr. Glade drew from the International Bibliographic Information on Dietary Supplements (IBIDS) Database that contains 691,193 scientific articles published in over 2,000 journals worldwide and several published texts. A total of 266 scientific publications were included in the preparation of his report.

Who Is Michael John Glade, Ph.D., F.A.C.N., C.N.S.?

Dr. Glade received his B.S., in Molecular Biology from the Massachusetts Institute of Technology and his Ph.D. from Cornell University. In addition he is a licensed dietician and certified nutrition specialist.

He has taught as an assistant professor, lecturer or visiting scientist at Rutgers University, the University of Maryland and Northwestern University Medical School.

He is currently the director and nutritionist advisor to the Board of Directors of the International College of Advanced Longevity Medicine as well as the President of the Certification Board for Nutrition Specialists.

Dr. Glade's research appears in many journals including the Journal of American Medical Association and the Journal of the American College of Nutrition. Here are only a few of his many publications:

Glade, M.J. 1997. Intake of dietary calcium to reduce the incidence of osteoporosis. Archives of Family Medicine 6: 491-494.

Glade, M.J. 1995. Management of disorders of cholesterol, triglyceride, and lipoprotein metabolism. Archives of Family Medicine 4: 869-878.

Glade, M.J., Y.S. Kanwar and P.H. Stern. 1994. Insulin and thyroid hormones alter chondrocyte metabolism in cell culture independently and in combination. Connective Tissue Research 31: 37-44.

Glade, M.J., Y.S. Kanwar and T.J. Hefley. 1991. Enzymatic isolation of chondrocytes from immature rabbit articular cartilage and their maintenance of phenotypic expression in culture. Journal of Bone and Mineral Research 6: 217-226.

Wright, L.L., M.J. Glade and J. Gopal. 1987. The use of transmission ultrasonics to assess bone status in the human newborn. Pediatrics Research 22: 541-544.

Dr. Glade helped prepare the glucosamine and chondroitin sulfate/osteoarthiritis risk reduction health claim petition and several others filed with the Food and Drug Administration.

Following is Dr. Glade's full review of the literature, his twenty-seven separate conclusions of the literature and his summary conclusion on Relief First capsules.

Composition of Articular Cartilage and the Biochemical and Physiologic Roles of *N*-Acetyl-D-Glucosamine and D-Glucosamine

Cartilage is composed of a complex extracellular matrix of collagen and elastic fibers within a hydrated gel of glycosaminoglycans and proteoglycans. This specialized network is stabilized by means of intermolecular and intramolecular cross-links that harness the swelling pressure exerted by the high concentration of negatively charged aggregates.[1] This accounts for more than 98% of the articular cartilage volume; cellular components constitute the remaining 2%. The interaction of these matrix components imparts the characteristic biomechanical properties of flexibility and resistance to compression. The matrix constituents undergo a distinct turnover process during which the catabolism and removal of molecules from the extracellular matrix is in balance with the synthesis and deposition of new molecules.[2]

Proteoglycans are large macromolecules consisting of multiple chains of glycosaminoglycan disaccharides and oligosaccharides attached to a central protein core.[3,4] The major disaccharide units of cartilage glycosaminoglycans are covalently bound by ($\beta 1 \rightarrow 4$) linkages and are formed by the ($\beta 1 \rightarrow 3$) linkage of either D-glucuronic acid or D-galactose to either *N*-acetyl-D-glucosamine or *N*-acetyl-D-galactosamine. The amino sugar residues are sulfated in either position 4 or 6.[3-5] The sulfate groups, together with the carboxyl groups of D-glucuronic acid, are ionized at tissue pH, conferring to the chain a strong global electronegative charge.[6-11] The presence of these negatively charged aggregates imparts to the matrix of articular cartilage its strong affinity for water and is the most significant contributor to the biomechanical properties of cartilage; in contrast, undersulfation of proteoglycans (such as during sulfur deficiency[12]) reduces their electronegative charge, water carrying capacity and mechanical loading limits.[13,14]

N-acetyl-D-glucosamine plays a pivotal role in cartilage metabolism and in cartilage matrix synthesis. This metabolite of D-glucosamine is the common precursor for the *de novo* biosynthesis of the individual glycosaminoglycans, keratan sulfate (via galactosyltransferase condensation of D-galactose and *N*-acetyl-D-glucosamine) and hyaluronan (via *N*-acetyl-glucosaminyltransferase condensation of D-glucuronic acid and *N*-acetyl-D-glucosamine).[15-22] These constituents of the cartilaginous matrix are almost all sulfated on their *N*-acetyl-D-glucosamine components[16] and their synthesis is absolutely dependent on the availability of sufficient amounts of *N*-acetyl-D-glucosamine.[15]

Endogenously, *N*-acetyl-D-glucosamine is produced by the enzymatic acetylation of its precursor, D-glucosamine-6-P, by D-glucosamine-6-P *N*-acetyltransferase.[15,23] D-glucosamine-6-P results from the enzymatic phosphorylation of exogenous D-glucosamine (2-amino-2-deoxyalpha-D-glucose) by hexokinase or via endogenous enzymatic conversion of D-glucose to D-glucose-6-P by glucokinase or hexokinase followed by the conversion of D-glucose-6-P to D-fructose-6-P by phosphoglucose isomerase and subsequently the conversion of D-fructose-6-P (plus L-glutamine) to D-glucosamine-6-P (plus L-glutamate) by L-glutamine-D-fructose-6-P amidotransferase (D-glucosamine-6-P synthase).[15,23-26]

However, the dependence of the articular cartilage extracellular matrix on the *de novo* synthesis of D-glucosamine and *N*-acetyl-D-glucosamine can be attenuated in the presence of abundant intrachondrocytic cytoplasmic *N*-acetyl-D-glucosamine, whether endogenous or exogenous in origin. In human chondrocytes, intracellular D-glucosamine and *N*-acetyl-D-glucosamine concentrations both are proportional to extracellular D-glucosamine and *N*-acetyl-D-glucosamine concentrations and the duration of cellular exposure to those concentrations.[15,23,27-29]

Chondroitin sulfate is a glycosaminoglycan that is polymerized into long, unbranched polysaccharide chains in which some of the constituent chondroitin moieties (produced by via *N*-acetyl-galactosaminyltransferase condensation of D-glucuronic acid and *N*-acetyl-D-galactosamine[17,20,30,31]) are sulfated on either position 4 or 6 of *N*-acetyl-D-galactosamine or position 2 of D-glucuronic acid.[5,32] Close control of chondroitin sulfate synthesis determines chain length, disaccharide composition and degree of sulfation, which vary with anatomic location, stage of development and age and are heterogeneous.[33-38] For example, the sulfation pattern of chondroitin disaccharides in normal human articular cartilage varies. The deeper layers of immature cartilage contain 4 times more sulfated residues than the upper regions of the immature tissue contain (as a result of polysulfation of some chondroitin residues in the extracellular matrix of the deeper regions).[33-35] All regions of the extracellular matrix of immature articular cartilage contain a smaller ratio of chondroitin-6-sulfate to chondroitin-4-sulfate than is typical of the extracellular matrix of articular cartilage in adults.[33-35] However, because at physiologic concentrations the intracellular conversions of D-glucosamine and *N*-acetyl-D-glucosamine to *N*-acetyl-D-galactosamine are very inefficient,[29,39,40] it is unlikely that exogenous D-glucosamine or *N*-acetyl-D-glucosamine can serve as precursors to endogenous *de novo* synthesis of chondroitin moieties.

Glycosaminoglycan polymers are secreted into the extracellular matrix covalently bound to proteins, forming protein-polysaccharide complexes called proteoglycans. In a proteoglycan, about 100 glycosaminoglycan chains, each containing 50 to 60 disaccharide units, are covalently attached to a polypeptide backbone composed of over 2,000 amino acids (the serine-rich core protein with a molecular weight of 250,000 to 300,000 daltons). The total molecular weight of an individual proteoglycan monomer is 1,500,000 to 2,500,000 daltons.[6]

One end of the core protein of a proteoglycan is non-covalently linked to a long polysaccharide filament of *N*-acetyl-D-glucosamine-containing hyaluronan through a link protein.[41-42] Approximately 100 core proteins are bound to an individual hyaluronan chain, forming a unit of aggrecan, the large molecular mass proteoglycan-hyaluronan aggregate predominant within the extracellular matrix of articular cartilage.[40,41]

The hydrodynamic properties of this aggregate determine the load-bearing capacity of articular tissue. As the electronegative charges of aggrecan draw water into the tissue, a large osmotic swelling pressure is created that swells and expands the extracellular matrix. This pressure produces tension within the interlacing collagen network of the matrix; balance is achieved when tension in the collagen network prevents further entry of water. Articular cartilage tissue swollen with water expresses substantial compressive resilience and offers considerable resistance to fluid flow and redistribution of water. Fully hydrated articular cartilage tissue behaves as a stiff

elastic polymer when exposed to sudden impact loading, with pressure-induced displacement of water from the matrix having little or no effect on matrix macromolecules (although sustained loads will produce slow inelastic deformation). Interstitial fluid pressurization during loading contributes more than 90% of load support, shielding the collagen-proteoglycan matrix from excessive stresses and reducing friction at the articular surfaces. Removal of loading allows re-entry of water and a return to the pre-loading high-tension equilibrium condition.[6,32,43-46]

Maturation and Aging of Articular Cartilage

In animals and humans, as infantile articular cartilage slowly matures into adult articular cartilage, the average size of matrix proteoglycans and the proportion of polysulfated chondroitin moieties within those proteoglycans gradually decrease, reducing the charge density, aggregability and water binding capacity of the articular cartilage tissue.[47-51] These trends continue as humans continue to age; in addition, the overall aggrecan content of the extracellular matrix of articular cartilage decreases as a result of decreasing total chondroitin sulfate contents of matrix proteoglycans and increasing incidence of defective core proteins in newly-synthesized proteoglycans.[52-57] Furthermore, the rate of degradative hydrolysis of matrix components accelerates; for example, unstimulated normal healthy human chondrocytes obtained from donors over 60 years of age secrete significantly greater amounts of matrix-degrading enzymes (especially stromelysin-1 and collagenase) and nitric oxide than are secreted by similar cells obtained from young adults.[58,59] Together these observations suggest that "aging" may imbalance matrix turnover in favor of degradative loss of healthy articular cartilage tissue.[58]

Events Culminating in Cartilage Degeneration and Mechanical Failure

Chronic imbalance in matrix macromolecule turnover producing net loss of articular tissue is a required precursor to the development of osteoarthritis and joint pain.[60,61] Numerous etiologic triggers can initiate the progression of events that eventually will culminate in tissue failure. For example, quadriceps muscle weakness significantly increases the risk for osteoarthritis in humans[62] and laxity in a joint may precede failure of the cartilage matrix.[63] A chronic imbalance of shock-absorbing and weight–bearing muscles affecting joint alignment[64,65] or overloading from excessive body weight[66] induces a mild yet chronic metabolic imbalance in the affected articular cartilage.

Regardless of the specific initiating factors, whenever mechanical compression of articular cartilage exceeds the tissue's load-bearing capacity, intrachondrocytic cyclo-oxygenase (COX) activity is stimulated.[67] Acceleration of COX activity results in increased intrachondrocytic production of prostaglandin E_2 (PGE_2),[68-71] an inducer of inducible nitric oxide synthase-2 (iNOS) activity.[67,72] Consequently, intrachondrocytic nitric oxide (NO) production is increased in proportion to the magnitude of compression and increasing local compression increases the recruitment of compression-responsive NO-producing articular chondrocytes.[67] Within articular chondrocytes, NO suppresses the synthesis of articular cartilage-specific type II collagen[73] and

stimulates the synthesis of nascent (inactive) IL-1β[74-76] and interleukin-1-converting enzyme (ICE).[77] ICE activates nascent inactive IL-1β.[76]

Activated IL-1β inhibits chondrocytic synthesis of collagen[74,75] and the expression of UDP-glucoronosyltransferase I mRNA, resulting in decreased synthesis of proteoglycans and their precursors,[74,75,78,79] while stimulating chondrocytic synthesis of matrix metalloproteinases (MMPs, including collagenases, gelatinases, aggrecanases, elastase, and fibronectin-degrading stromelysin-1)[58,72,80-94] and growth-related oncogene-α (GRO-α), a stimulator of stromelysin-1.[95] During continued activation, IL-1β itself stimulates increased COX-2 activity and production and secretion of NO and PGE_2.[59,72,78,82,96-102] Together, these responses establish a cooperative positive feedback cycle that reinforces the progression of metabolic alterations to osteoarthritis.[103,104] The accumulation of collagenase-generated collagen type II breakdown products is accompanied and may be preceded by denaturation of the triple helix of type II collagen and defibrillation of the collagen fibril network, with loss of tensile strength and ability to resist the swelling pressure that is exerted by the hydration of the polyanionic proteoglycan aggregates of the extracellular matrix.[83-86] The resulting attenuation of synthetic activity combined with acceleration of degradation of extracellular matrix components results in net decreases in the absolute amounts of aggrecan, type II collagen and other matrix macromolecules present within the articular cartilage tissue as well as in the mechanical resilience of the tissue.[60,61,86,105]

Abnormal mechanical loading also stimulates chondrocyte and synoviocyte secretion of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).[60,61] IL-1β, IL-6, TNF-α and nitric oxide suppress differentiated matrix synthetic activity and stimulate replicative clonal expansion of chondrocytes ("reactive proliferation").[93,106-108] During early metabolic imbalance in articular cartilage, these clones are composed of daughter cells that express predominantly a "fetal" differentiation pattern characterized by the production of abnormally large and abnormally sulfated chondroitin sulfate polymers that resist incorporation into proteoglycans and of significantly smaller-than-normal-for-age chondroitin sulfate chains that become incorporated into a population of newly-synthesized proteoglycans that then are relatively deficient in sulfate content; these metabolic shifts result in a significant depletion of normally sulfated proteoglycans of normal molecular sizes and a significantly decreased total glycosaminoglycan content.[108-121] Both the abnormally small proteoglycans and the abnormally large proteoglycans are less able to aggregate with hyaluronan to form aggrecan, resulting in a significantly decreased average size of aggrecan macromolecules and a significantly increased proportion of nonaggregated proteoglycans.[122] The "replacement matrix" exhibits increased synthesis of more readily hydrolyzable (easily degradable) collagens[123,124] and resembles fibrous cartilage[125] or nonosteoarthritic human articular cartilage after partial enzymatic hydrolysis.[106]

Mechanical loading beyond the load-bearing capacity of load-bearing articular cartilage also stimulates the expression of N-cadherin, intercellular adhesion molecule-3 (ICAM-3), neural cell adhesion molecule (NCAM) and vascular cell adhesion molecule-1 (VCAM-1).[126] In addition, the increasingly abnormal matrix often is fibronectin-deficient as a result of the fibronectin-

degrading activity of stromelysin-1.[60,61,78] Loss of cell adhesion molecules impairs chondrocyte anchorage and produces anoikis (cell death resulting from loss of normal cell-substratum contact).[60,61,126,127] IL-1β-induced direct activation and NO-induced indirect activation of pro-apoptotic nuclear factor κB (NF-

κB) further contribute to loss of cellularity within the articular cartilage tissue.[79,127-130] Within the increasingly hypocellular tissue, the inferior undersulfated extracellular matrix[131] exhibits compromised ability to regain metabolic equilibrium, decreased water binding capacity, inferior biomechanical competence and functional incompetence that render it prone to fibrillation and mechanical failure.[60,61,79,125,126,132-137]

Chronic Degeneration of the Extracellular Matrix of Articular Cartilage is a Required Precursor to Osteoarthritis

Changes in the macromolecular composition of the extracellular matrix of articular cartilage are characteristic of clinically apparent osteoarthritis. Osteoarthritic rat articular cartilage, compared to nonosteoarthritic articular cartilage, exhibits significantly decreased total proteoglycan, chondroitin 4-sulfate and chondoitin 6-sulfate contents and significantly increased stromelysin-1 (fibronectin-degrading) activity.[138] In addition, the percentage of apoptotic chondrocytes in the tissue is significantly increased.[138] Proteoglycans in osteoarthritic adult bovine articular cartilage are larger than normal adult bovine articular cartilage proteoglycans (with larger chondroitin sulfate polymers) and closely resemble proteoglycans found in the articular cartilage matrix of calves.[5] Osteoarthritic equine articular cartilage contains a significantly increased proportion of unsulfated disaccharides and a significantly decreased proportion of chondroitin 6-sulfate.[139] The articular cartilage of Cynomolgus macaque monkeys with arthritis exhibits increased production of abnormal chondroitin sulfate-containing polymers.[140]

In degenerative joint disease in dogs, affected articular cartilage contains significantly increased amounts of newly-synthesized large chondroitin sulfate-rich and glucosamine- and galactosamine-poor proteoglycans typical of those produced by immature canine articular cartilage.[115,141,142] As cartilage degeneration progresses, affected canine articular cartilage exhibits significantly increased production of abnormal chondroitin sulfate-containing polymers, significantly increased water content, significantly increased proteoglycan content, significantly increased percentage of smaller proteoglycans and significantly decreased percentage of chondroitin sulfate in proteoglycans.[111,143,144] Some newly synthesized proteoglycans are abnormally large (containing abnormally long chondroitin sulfate chains) and a second population of proteoglycans are abnormally small; both have lost the ability to aggregate spontaneously with hyaluronan, compromising the hydrodynamic properties of the tissue.[145]

Compared to non-diseased tissues, the extracellular matrix of articular cartilage harvested from osteoarthritic human joints resembles the extracellular matrix of osteoarthritic articular cartilage harvested from other species. Matrix glycosaminoglycans, proteoglycans, aggrecans and collagen are relatively depleted while the aggrecan that is present is characterized by significant

undersulfation.[146-150] Nonetheless, the responses of chondrocytes within osteoarthritic articular cartilage to cytokine and nutritional stimulators are not different from those of of chondrocytes within nonosteoarthritic articular cartilage.[73,151,152]

Pathologic changes in cartilage matrix composition and organization alter the affinity of the matrix for water and produce excessive cartilage deformation under loading.[153,154] When chronic, the excessive tissue deformation induces adaptive structural and compositional changes that confer increased stiffness in the tissue,[64] increasing its vulnerability to the compressive, tensile and shear forces that occur during normal joint function.[32,137] Grossly apparent cartilage erosion does not appear until the tissue has lost considerable stiffness and is undergoing progressive mechanical failure.[64]

As a result of the changes occurring in articular cartilage, abnormally transmitted mechanical stress produces microfractures within the tissue matrix that in turn increase the stresses on surrounding tissue and induce increased chondrocyte secretion of metalloproteinases.[137,155] The subsequent enzymatic tissue degradation potentiates local tissue stress and initiates a positive feedback loop. Increased loading on subchondral bone stimulates the attempt to reduce mechanical stress by increasing joint surface area through the production of bone spurs (osteophytes) at the joint margins (which confer the hard bony enlargement that is characteristic of chronic osteoarthritis).[155]

The Culmination of Matrix Degeneration in Clinical Osteoarthritis

In the US, the incidence of at least one joint with osteoarthritis among those aged 15 to 40 years is about 5%; this increases to over 60% among those over 65 years old.[156] Overall, the prevalence of at least mildly symptomatic osteoarthritis in at least one joint is about 30%.[157] Symptomatic osteoarthritis of the knee occurs in about 6% of US adults aged 30 years and older,[158] although radiographic changes of the femorotibial compartment occur in 5% to 15% of people aged 35 to 74 years.[159]

Clinical osteoarthritis (also known as degenerative joint disease) is characterized by focal loss of cartilage and hypertrophic bone spurs.[155] Although the term osteoarthritis refers to the overgrowth of bone at the margins and subchondral areas of the joint, and despite the eventual bony involvement in later stages of the disease, osteoarthritis is marked by net loss of cartilage tissue. Initial loss of articular cartilage tissue is mild but may progress to full thickness erosions and eventual bone-to-bone contact (loss of all joint space). Narrowing of the joint space may reflect other degenerative changes in addition to articular cartilage erosion;[160] as cartilage degeneration progresses, subchondral bone density and volume increase (consistent with increased transmission of load bearing into the subchondral bone).[161]

The primary complaint in osteoarthritis is pain, particularly upon use of the affected joint.[155] Pain can be accompanied by varying degrees of joint stiffness, limitation of movement,

tenderness and swelling at the joint margins and loss of function. Osteoarthritis often is asymmetric. There are no systemic symptoms outside the affected joint.[155]

Possible causes of pain in human osteoarthritis include osteophyte growth with stretching of the periosteum, increased intraosseous pressure, microfractures, ligament damage, capsular tension, meniscal injury and synovitis.[162] Radiologically measured decrease in joint space is significantly correlated with increase in pain severity, although the clinical utility of pain assessment as an estimator of joint deterioration is under debate.[163]

Bioavailability of Supplemental D-Glucosamine and *N*-Acetyl-D-Glucosamine

D-Glucosamine: There are 3 forms of commercially-available D-glucosamine: D-glucosamine (MW: 179), D-glucosamine-HCl (MW: 270) and D-glucosamine sulfate (a derivative of the naturally occurring cartilage extracellular matrix constituent, aminomonosaccharide D-glucosamine;[164] MW: 456). Because of the differences in molecular size, 1500 mg of D-glucosamine-HCl provides as much D-glucosamine as is provided by 2600 mg of D-glucosamine sulfate or 1040 mg of D-glucosamine. A daily intake of 1500 mg of D-glucosamine sulfate is equivalent to a daily intake of between 15 and 30 mg/kg body weight.

In studies in rats, 90% to 95% of ingested D-glucosamine sulfate was absorbed intact into the blood and about 3% of newly absorbed D-glucosamine sulfate was incorporated into newly synthesized proteoglycans in articular cartilage tissues.[165,166] In studies in humans, consumption of 314 mg of crystalline D-glucosamine sulfate was followed by the absorption of about 280 mg (about 90%) intact into the bloodstream; about 50% of this amount (about 140 mg) survived hepatic first-pass extraction intact.[167] When the consumption of 1884 mg occurred as one bolus or in three divided intakes of 626 mg every 4 hours, there was no difference in total D-glucosamine sulfate bioavailability to systemic tissues (about 40% to 50% of the amount ingested). Other investigators have reported that over 90% of ingested D-glucosamine sulfate was absorbed intact into the human enterohepatic circulation.[168,169] One investigator reported that about 75% of ingested D-glucosamine sulfate was bioavailable to body tissues following hepatic first-pass extraction.[169]

The plasma D-glucosamine concentration in naïve adults has been reported to be approximately 0.055 μg/ml (0.3 μM).[170] This concentration increases nonlinearly with increasing oral intake of D-glucosamine.[170] There is some disagreement concerning the mean peak plasma concentrations that are observed following oral consumption of D-glucosamine.[168,170-172] In one study, mean peak plasma concentrations were reported to be 1.07 μg/ml (5.9 μM), 1.6 μg/ml (8.8 μM) and 2.5 μg/ml (13.8 μM) at 3 to 4 hours following the acute consumption of 750 mg, 1500 mg and 3000 mg of D-glucosamine sulfate, supplying 588 mg, 1175 mg and 2350 mg of D-glucosamine, respectively.[170] In another study, the acute consumption of 1500 mg of D-glucosamine sulfate (supplying 1175 mg of D-glucosamine) produced peak plasma concentrations of D-glucosamine that ranged from 0.34 to 2.0 μg/ml (1.9 to 11.0 μM).[171] In yet another study, the acute consumption of 1500 mg of D-glucosamine sulfate (supplying 1175 mg of D-glucosamine)

produced peak plasma concentrations of D-glucosamine that ranged from 3.0 to 3.3 µg/ml (16.5 to 18.2 μM).[172] Other investigators reported that the acute consumption of 6000 mg of D-glucosamine sulfate (supplying 4700 mg of D-glucosamine) produced peak plasma concentrations of D-glucosamine that were less than 10 µg/ml (55 μM).[168] It also has been estimated that a plasma D-glucosamine concentration of 2.5 µg/ml (13.8 μM) would produce an interstitial (extracellular) D-glucosamine concentration in articular cartilage tissue of 1.0 µg/ml (5.5 μM) and an intrachondrocytic D-glucosamine concentration of 0.25 µg/ml (1.4 μM).[29]

In healthy subjects, ingestion of D-glucosamine sulfate was followed by increased serum sulfate concentration. In contrast, ingestion of sodium sulfate did not effect serum sulfate concentration, suggesting that dietary supplementation with D-glucosamine sulfate might provide D-glucosamine, free sulfate and D-glucosamine sulfate for proteoglycan synthesis.[173]

N-Acetyl-D-Glucosamine: There have been estimates that the absorption of *N*-acetyl-D-glucosamine is about half as efficient as is the absorption of D-glucosamine.[174] In one report, the consumption of 1000 mg of *N*-acetyl-D-glucosamine increased plasma *N*-acetyl-D-glucosamine concentration from 10 to 25 μM in 2 to 4 hours post-consumption.[175,176]

Biochemical and Physiologic Roles of *N*-Acetyl-D-Glucosamine in the Preservation of Articular Cartilage

Equine nonosteoarthritic articular cartilage explants in organ culture cultured in the presence of *N*-acetyl-D-glucosamine ($1500~\mu M$) exhibited little ability to prevent LPS-induced increases in nitric oxide production and proteoglycan degradation, although the rate of synthesis of new proteoglycans was increased significantly.[177] In cultures of chondrocytes harvested from nonosteoarthritic human articular cartilage, the presence of *N*-acetyl-D-glucosamine ($1000~\mu M$) significantly stimulated cell proliferation and ($500~\mu M$) significantly stimulated the incorporation of $^{35}SO_4$ into newly-synthesized poroteoglycans.[178] In another report, the addition of *N*-acetyl-D-glucosamine to the culture media of chondrocytes harvested from nonosteoarthritic human articular cartilage produced significant attenuation of IL-1β-induced production of NO ($2500~\mu M$ but not $1500~\mu M$) and significant attenuation of IL-1β-induced COX-2 activity ($10000~\mu M$).[179]

Although the scientific literature regarding the direct dietary supplementation with *N*-acetyl-D-glucosamine is sparse, because the major intrachondrocytic metabolic fate of supplemental D-glucosamine is conversion to *N*-acetyl-D-glucosamine,[15,23-26] and circulating *N*-acetyl-D-glucosamine is readily taken up by chondrocytes,[15,23,27-29] the effects of dietary supplementation with D-glucosamine (in any of its three forms: D-glucosamine, D-glucosamine HCl and D-glucosamine sulfate) can be expected to be mimicked by dietary supplementation with *N*-acetyl-D-glucosamine.

Biochemical and Physiologic Roles of D-Glucosamine in the Preservation of Articular Cartilage

In cultures of chondrocytes harvested from nonosteoarthritic rat articular cartilage, the addition of D-glucosamine ($5500~\mu M$[78] or $25000~\mu M$[79]) to the culture medium prevented IL-1β-induced inhibition of the expression of UDP-glucuronosyltransferase I mRNA and of proteoglycan synthesis, as well as IL-1β-induced activation of pro-apoptotic nuclear factor κB (NF-κB). Similarly, D-glucosamine ($1500~\mu M$) significantly inhibited the secretion of collagenase by cultured nonosteoarthritic equine articular chondrocytes.[180] In contrast, crystalline D-glucosamine sulfate ($55~\mu M$[181] or $500~\mu M$[178] D-glucosamine) stimulated the production of proteoglycans by chondrocytes harvested from nonosteoarthritic human articular cartilage in cell culture while ($2500~\mu M$ but not $1500~\mu M$) significantly attenuated IL-1β-induced production of NO.[179]

When added to the culture media of chondrocytes harvested from osteoarthritic human articular cartilage, crystalline D-glucosamine sulfate ($50~\mu M$,[182] $100~\mu M$,[183] $2000~\mu M$[184] or $5500~\mu M$[72] of D-glucosamine) inhibited the inherent and IL-1β-induced catabolic activity of metalloproteases secreted by the chondrocytes and stimulated the synthesis of physiologically-relevant proteoglycans with chemical characteristics of proteoglycans synthesized by chondrocytes harvested from nonosteoarthritic human articular cartilage. Similarly, when added to the culture media of chondrocytes harvested from osteoarthritic human articular cartilage, crystalline D-

glucosamine sulfate (550 μM of D-glucosamine) significantly attenuated IL-1β-induced increase in NF-κB activity; a ten-fold higher concentration (providing 5500 μM of D-glucosamine) significantly attenuated IL-1β-induced increases in COX-2 gene expression and activity and PGE$_2$ synthesis and secretion.[185] When added to the culture media of chondrocytes harvested from osteoarthritic human articular cartilage, in which adhesion of chondrocytes to fibronectin and overall protein synthesis are significantly inhibited while extracellular collagenase activity is significantly increased, D-glucosamine (50 μM[127] or 50 μM[183] D-glucosamine) restored the adhesive properties of the chondrocytes, significantly reduced extracellular collagenase activity and significantly increased the rate of protein synthesis.

In organ culture systems, both D-glucosamine-HCl (275 μM) and D-glucosamine sulfate (275 μM D-glucosamine) added to the culture medium of nonosteoarthritic rat femoral articular cartilage explants in organ culture significantly increased the rates of collagen and proteoglycan synthesis and partially prevented nonsteroidal anti-inflammatory drug- (NSAID)-induced inhibition of proteoglycan synthesis.[25]

The addition of D-glucosamine-HCl (250 μM[135] or 1400 μM[136]) to the culture medium of nonosteoarthritic equine articular cartilage explants in organ culture prevented IL-1β-induced increases in the activities of stromelysin-1, collagenase and gelatinase and bacterial lipopolysaccharide (LPS)- and IL-1β-induced increases in the production of NO and PGE$_2$ and the degradation of extracellular matrix proteoglycans. In the same nonosteoarthritic equine organ culture system, D-glucosamine-HCl significantly attenuated LPS-induced PGE$_2$ secretion (2750 μM) and in higher concentration (5500 μM) significantly attenuated LPS-induced collagenase secretion and glycosaminoglycanolysis.[186] Although in this system lower concentrations of D-glucosamine were unable to attenuate the pro-oateoarthritic actions of IL-1β and LPS, in the absence of these stimulators, D-glucosamine (14 μM) stimulated the incorporation of $^{35}SO_4$ into newly-sythesized matrix glycosaminoglycans while inhibiting basal glycosaminoglycanolysis.[187]

When D-glucosamine was added to the culture medium of nonosteoarthritic bovine articular cartilage explants in organ culture in a concentration (2500 μM) that significantly inhibited IL-1β-induced aggrecanase cleavage of aggrecan, lactate production was unaffected and D-glucosamine was incorporated into newly-synthesized chondroitin sulfates,[188] indicating that the inhibition of IL-1β-induced catabolism was not an artefact of D-glucosamine-induced general inhibition of chondrocyte cellular metabolism.[184] As in articular cartilage tissues obtained from other species, D-glucosamine-HCl also stimulated sulfate incorporation into chondroitin sulfates (55 μM[189]) and attenuated LPS-induced NO production (2750 μM[190]), LPS-induced proteoglycan degradation (5500 μM[190]), retinoic acid-induced degradation of aggrecan (2000 μM[191]) and IL-1β-induced expression and secretion of inducible nitrix oxide synthase, COX-2 and PGE$_2$ (27.5 μM[100]) in the extracellular matrix of nonosteoarthritic bovine articular cartilage explants in organ culture.

Osteoarthritic articular cartilage tissue samples harvested from rabbits that had been fed diets supplemented with D-glucosamine-HCl (20 mg/kg body weight daily; approximately equivalent

to a daily human intake of 1500 mg of D-glucosamine-HCl) exhibited significantly accelerated rates of synthesis of new proteoglycans and significant deceleration of matrix proteoglycan loss compared to articular cartilage tissue samples harvested from unsupplemented animals.[192,193]

Supplemental D-Glucosamine and Clinical Osteoarthritis

Dietary supplementation with D-glucosamine-HCl (2000 mg/day) produced a significantly greater decrease in subjective pain assessment in 12 weeks than did placebo in adults with regular knee pain that had not yet progressed to clinically identifiable osteoarthritis.[194] However, there were no significant differences in the improvement in clinical or functional tests of joint motion and balance. In contrast, dietary supplementation with D-glucosamine-HCl (1500 mg/day) for 2 to 4 months was no better than the consumption of placebo in relieving joint pain in individuals with mild to severe femorotibial osteoarthritis.[195,196] However, in at least one of these studies,[196] subjects were alternated between D-glucosamine-HCl and D-glucosamine sulfate and the placebo also was changed during the study, diminishing any value of that report.

On the other hand, in 2 randomized double-blind placebo-controlled clinical studies, compared to the effects of placebo, dietary supplementation of subjects with mild to severe femorotibial osteoarthritis with crystalline D-glucosamine sulfate (Dona®; 1500 mg/day) for 1 month has produced significantly greater reductions in articular pain, tenderness, swelling and restriction of movement.[197,198] In another study, short-term dietary supplementation with Dona® (1500 mg/day) 4 weeks produced a significantly greater decrease in the Lequesne functional index of impairment and a significantly greater increase in the percentage of "responders" (subjects experiencing a decrease of at least 3 points in the Lequesne index) than did placebo.[199] Compared to subjects consuming placebo, subjects consuming D-glucosamine sulfate experienced no differences in the incidence or severity of side effects or in the results of routine clinical chemistry, hematology, urinalysis, heart rate, blood pressure or body weight. Similarly, dietary supplementation with Dona® (1500 mg/day) for 6 to 8 weeks produced significantly greater decreases in joint pain, tenderness and swelling and in the number of days until improvement was noted in joint pain, tenderness or swelling as well as significantly greater increase in the percentage of patients experiencing some degree of improvement in joint pain, tenderness or swelling without producing differences in the incidence or severity of side effects or in hematologic or urinary variables, compared to the effects of placebo consumption.[200]

In long-term randomized double-blind placebo-controlled clinical studies, compared to the effects of placebo, 3 years of dietary supplementation with Dona® (1500 mg/day) by subjects with mild to severe femorotibial osteoarthritis produced significantly greater reductions in the mean rate of femorotibial joint space narrowing (measured as the width of the medial femorotibial joint space, with the knee in full extension, by visual inspection; the "preferred, gold standard outcome" in studies of osteoarthritis[201]), the WOMAC total pain score, the WOMAC indices of total knee health, pain, function and stiffness, the Lequesne functional index and pain assessed by a visual analog scale.[201-204] In addition, the number of subjects experiencing "severe" (i.e., > 0.5 mm) joint space narrowing was significantly smaller after 3

years of dietary supplementation with D-glucosamine sulfate. However, among those subjects consuming D-glucosamine sulfate, those with less severe osteoarthritis at baseline tended to experience better responses. Furthermore, 3 years of daily dietary supplementation with 1500 mg of D-glucosamine sulfate produced no greater number or severity of side effects, including changes in the results of routine annual clinical laboratory examinations, than did 3 years of consumption of placebo.

In a far-ranging multicenter open-label study, a total of 1208 evaluable subjects were supplemented with Dona® (1500 mg/day) for 13 to 99 days.[205] Physician ratings of subject responses were highly favorable: "good" (59% of subjects), "sufficient" (36% of subjects) and "insufficient" (5% of subjects). The best response was experienced by subjects with osteoarthritis of the knee or elbow, while those with osteoarthritis of the hip fared more poorly. The effect of D-glucosamine sulfate on pain scores was directly proportional to the duration of supplementation. In a more targeted open-label study, 69 young athletes (mean age 19 years) with cartilage degeneration of the knee (biochemically similar to osteoarthritis) received dietary supplementation with Dona® (1500 mg/day for 4 days, then 750 mg/day for 90 to 120 additional days.[206] After 120 days, complete remission of symptoms (patella-grinding sound, patella-displacement pain, patella-pressure pain) was reported for 76.5% of the subjects.

In two uncontrolled studies, subjects with femorotibial osteoarthritis were supplemented with either Dona® (1500 mg/day) or ibuprofen (1200 mg/day) for 4 weeks.[207,208] In both studies, both groups experienced similar significant decreases in the Lequesne index of functional impairment[207] and in pain at rest, pain during movement, pain under loading and joint swelling[208] (compared to baseline). However, in both studies there were significantly more adverse events and adverse event-related trial dropouts among the subjects consuming ibuprofen. In a similar uncontrolled study, subjects with femorotibial osteoarthritis were supplemented with either Dona® (1500 mg/day) or ibuprofen (1200 mg/day) for 8 weeks.[209] In this study, Dona® produced a significantly greater decrease in subjective assessment of knee pain with no difference in the incidence or severity of side effects.

Among subjects with osteoarthritis of the temporomandibular joint, D-glucosamine sulfate (Jamieson™; Windsor, Ontario, Canada; 1500 mg/day for 90 days) supplementation produced a significantly greater decrease in pain assessed using a visual analog scale compared to the pain relief afforded by ibuprofen (1200 mg/day for 90 days).[210] There were no significant differences between D-glucosamine sulfate and ibuprofen in the production of significant reduction in masticatory muscle pain and significant increases in pain-free mouth opening and voluntary mouth opening.

Several groups of investigators have applied the techniques of meta-analysis to evaluate dietary supplementation with D-glucosamine sulfate. One group concluded that the randomized double-blind placebo-controlled studies of adequate quality to include in their analysis demonstrated that dietary supplementation with D-glucosamine sulfate (1500 mg daily for at least 6 weeks) produced significantly greater reductions in the Lequesne Index of functional impairment, the severity of pain assessed using a visual analog scale and voluntary consumption of NSAID's for

rescue from pain than did placebo (the effect sizes were "large").[211,212] In addition, it was concluded that D-glucosamine sulfate has demonstrated a consistently excellent safety profile.

Other investigators concluded that dietary supplementation with D-glucosamine sulfate by individuals with osteoarthritis consistently produced significant decreases in joint pain and significant increases in joint function of small-to-moderate magnitude,[213] that dietary supplementation with D-glucosamine sulfate is "probably effective in osteoarthritis in reducing pain and in improving joint function"[159] and that dietary supplementation with D-glucosamine sulfate (1500 mg/day) produces significantly increased pain relief in individuals with femorotibial osteoarthritis accompanied by an excellent safety profile.[214]

When only "high quality" studies were considered by other investigators, it was concluded that dietary supplementation with D-glucosamine sulfate by individuals with osteoarthritis produced approximately 50% reductions in pain and risk for disease progression with a similar degree of improvement in function (a "large" effect consistently greater than that of placebo).[215,216] However, it was noted that the quality of most published studies concerning dietary supplementation with D-glucosamine sulfate by individuals with osteoarthritis has been generally poor and that the magnitude of the reported effects of dietary supplementation with D-glucosamine sulfate are likely to be inflated by weaknesses in the study designs and analysis. Nonetheless, it was concluded that, despite their poor flaws, the available published studies demonstrate a significant degree of efficacy for dietary supplementation with D-glucosamine sulfate.[215,216]

Daily Intakes of Supplemental *N*-Acetyl-D-Glucosamine and D-Glucosamine that are Effective in Reducing the Symptoms of and Risk of Developing Osteoarthritis

The reliable and credible scientific literature indicates that daily dietary supplementation with 1500 mg of either D-glucosamine or D-glucosamine sulfate is effective in reducing the symptoms of and risk of developing osteoarthritis. Although not adequately tested as a treatment for osteoarthritis in human clinical trials, *N*-acetyl-D-glucosamine, by virtue of its position as the biochemical intermediary through which supplemental D-glucosamine must act, and its somewhat greater bioavailability, is likely to express potency similar to that of D-glucosamine and D-glucosamine sulfate.

Supplemental *N*-Acetyl-D-Glucosamine and Rheumatoid Arthritis

Rheumatoid arthritis is an inflammatory arthritis afflicting synovial joints that is characterized by primary synovitis, proliferative thickening of the joint capsule and secondary degeneration of bone and joint with eventual erosion of the joint surfaces.[217-219] Commonly perceived to be an autoimmune disorder,[220] rheumatoid arthritis appears to be triggered by the excessive exposure of the synovium to IL-1β.[221,222] Once the articular cartilage surface becomes exposed to excess of this pro-inflammatory cytokine, the pathogenesis of the cartilaginous involvement in rheumatoid arthritis resembles that of osteoarthritis.[222]

The induction of rheumatoid arthritis-like arthropathy in rodents through the expedient of intra-articular injections of irritating adjuvant solutions has become a commonly-studied model of human rheumatoid arthritis.[223]   In this model, one of the first events in response to a stimulus is the release of IL-1β by the synovial membrane.[224,225]   Compared to the effects of placebo, rodents with established adjuvant-induced arthropathy and later supplemented with oral D-glucosamine (as D-glucosamine-HCl[223] or as D-glucosamine sulfate[226]) daily for several weeks have exhibited significant reductions in joint swelling, lameness, synovial hyperplasia, loss of cartilage extracellular matrix substance, inflammatory cell infiltration of joint spaces and plasma concentrations of NO, PGE_2 and IL-1β. Interestingly, presupplementation with D-glucosamine sulfate (50 mg per kg body weight daily) conferred to rats resistance to subsequent attempts to induce tibio-tarsal arthritis via intra-articular injections of kaolin or adjuvant.[227]

Although not adequately tested as a treatment for rheumatoid arthritis in human clinical trials, *N*-acetyl-D-glucosamine, by virtue of its position as the biochemical intermediary through which supplemental D-glucosamine must act, and its somewhat greater bioavailability, is likely to express potency similar to that of D-glucosamine and D-glucosamine sulfate in the amelioration of the symptoms of rheumatoid arthritis.

Supplemental *N*-Acetyl-D-Glucosamine and Gout

Gout is characterized by acute attacks of painful edematous arthritis that are triggered by the local precipitation of monosodium urate crystals into the joint spaces.[228-230]   These crystals act as stimuli for secretion of the pro-inflammatory cytokine, 5(*S*),12(*R*)-dihydroxy-6,8,10-(*trans/trans/cis*)-14-*cis*-eicosatetraenoic acid (leukotriene B_4; LTB_4) by peripheral polymorphonuclear leukocytes.[228,231-233] LTB_4 stimulates neutrophil and eosinophil chemotaxis, chemokinesis, release of lysosomal enzymes, and generation of superoxide with overall increased inflammatory activity in the synovium.[232,233]   In addition, LTB_4 stimulates the secretion of arthritogenic IL-1β by the synovial membrane, contributing to the initiation of arthropathy.[234]

Although not adequately tested as a treatment for gout in human clinical trials, *N*-acetyl-D-glucosamine is likely to be effective in the treatment of any IL-1β-induced arthropathy, including gout.

Supplemental *N*-Acetyl-D-Glucosamine and Inflammation

N-acetyl-D-glucosamine has been reported to significantly attentuate the secretion of pro-inflammatory mediators by activated human polymorphonuclear leukocytes[235,236] and lymphocytes.[237,238]

D-glucosamine appears to act by interrupting message transduction. Following transport across the chondrocyte cell membrane by the GLUT-2 and GLUT-4 glucose transporters,[239,240] supplemental D-glucosamine stimulated the expression of IL-1 cell membrane receptor subtype II, which binds IL-1β with high affinity but produces an inactive receptor-ligand complex, effectively intercepting IL-1β-based signal transmission.[79] In other cell culture models, D-glucosamine-HCl (0.01 to 1.0 mM) dose-dependently suppressed the superoxide anion generation induced by formyl-Met-Leu-Phe (fMLP) or complement-opsonized zymosan and inhibited the phagocytosis of complement-opsonized zymosan or IgG-opsonized latex particles.[241] Similarly, D-glucosamine-HCl significantly inhibited fMLP-induced up-regulation of CD11b, polymerization of actin, and activation via phosphorylation of pro-apoptotic p38 mitogen-activated protein kinase (MAPK).[241] In addition, D-glucosamine-HCl inhibited the release of lysozymes from phagocytozing neutrophils and suppressed neutrophil chemotaxis toward zymosan-activated serum.[241] Furthermore, supplemental D-glucosamine inhibited the activation of T-lymphocytes and the reactivity of leukocytes without producing signs of cellular toxicity.[237] All of the effects of supplemental D-glucosamine provide evidence that its anti-inflammatory, anabolic and anticatabolic properties result at least in part from interaction with intercellular and intracellular cytokine-based communication systems.

Safety of Effective Daily Intakes of Supplemental *N*-Acetyl-D-Glucosamine and D-Glucosamine

N-acetyl-D-glucosamine and D-glucosamine arepresent in all foods containing cartilage or glycoproteins.[14] Neonatal rat femnoral condyles have exhibited no cytotoxic responses to exposure to 25000 μM of *N*-acetyl-D-glucosamine.[107] Rats that have consumed up to 2800 mg of *N*-acetyl-D-glucosamine per kg of body weight for 13 weeks have exhibited no effects on body weight, rate of weight gain, food intake, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin content, platelet count, white blood cell count, percentages of segmented leukocytes, eosinophils, basophils, lymphocytes, monocytes or reticulocytes, plasma concentrations of hemoglobin, total protein, albumin, total bilirubin, total cholesterol, urea-nitrogen, creatinine, calcium, phosphorus, sodium, potassium or chloride, plasma activities of γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) or alkaline phosphatase or weights or gross or histologic morphologies of brain, thymus, lungs, heart, spleen, liver, adrenal glands, kidneys or testes.[242]

It has been possible to estimate an LD_{50} for oral D-glucosamine of 8000 mg per kg of body weight,[243] although no deaths have occurred in mice and rats acutely consuming up to 5000 mg per kg of body weight[244] or 2700 mg per kg of body weight daily for 12 months.[243] Daily dietary supplementation with 2149 mg of D-glucosamine sulfate per kg body weight produced no systemic or gastrointestinal adverse reactions in dogs.[227] Horses fed 8 g of D-glucosamine-HCl daily for 48 weeks (equivalent to about 16 mg/kg body weight daily in an adult human) exhibited no effects on bone metabolism.[245] In humans, intra-articular (200 mg)[246] or intramuscular injection of D-glucosamine sulfate (200 mg once[246] or 400 mg daily for 7 days[247,248]) produced no adverse reactions. Dietary supplementation with D-glucosamine sulfate[196-204,249] or D-

glucosamine-HCl[193-196,250-252] for up to 3 years did not produce an increase in the incidence or severity of side effects in placebo-controlled human studies.

N-acetyl-D-glucosamine is sufficiently safe to be recommended as a replacement osmotic solute in peritoneal dialysis fluids.[253] The most common side effects reported by humans consuming D-glucosamine sulfate include reversible epigastric pain, epigastric tenderness, heartburn, nausea, diarrhea, dyspepsia, vomiting, constipation, drowsiness, headaches, and mild skin reactions.[205,208] Oral D-glucosamine sulfate does not interfere with the efficacy of medications for cardiovascular, liver, or lung diseases, diabetes or depression,[247] interfere with intracellular glucose metabolism (when present in concentrations at all possible to achieve even with massive amounts of oral intake)[27,254-260] or produce insulin resistance in rats[28,261,262] or humans.[179,201,244,263-267] However, oral D-glucosamine sulfate may potentiate active peptic ulcers.[205] Obesity may reduce responsiveness to dietary supplementation with D-glucosamine sulfate.[205]

Conclusions

- Maintaining the structural and functional integrity of the proteoglycan component of the extracellular matrix of articular cartilage is required for preservation of healthy joint architecture and biomechanics.

- Imbalanced metabolism favoring catabolism within the extracellular matrix of articular cartilage produces degenerative changes in the proteoglycan composition of the matrix.

- Compromise of the structural and functional integrity of the proteoglycan component of the extracellular matrix of articular cartilage results in net loss of articular cartilage tissue, inferior biomechanical competence and structural deformation of joint architecture.

- Net degradation of the extracellular matrix of articular cartilage, accompanied by the production of spontaneous repair matrix with abnormal proteoglycan composition, results in asymptomatic subclinical osteoarthritic change.

- The initiation of asymptomatic osteoarthritic change is not inevitable.

- The progression of asymptomatic osteoarthritic change to osteoarthritis is not inevitable.

- The progression of osteoarthritic change is required in order for abnormalities in articular cartilage composition and structure to progress to osteoarthritis.

- Osteoarthritic change in the absence of joint pain represents a modifiable risk factor for later development of osteoarthritis.

- The progression of symptomatic osteoarthritis is not inevitable.

- Rheumatoid arthritis is an arthropathy triggered by excessive exposure of articular cartilage and synovial tissues to pro-inflammatory cytokines.

- Gout is a form of arthritis with a pathogenesis that requires excessive exposure of articular cartilage and synovial tissues to pro-inflammatory cytokines.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can attenuate the catabolic effects of pro-inflammatory and degradative stimuli on articular cartlage.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can enhance the synthetic activity within articular cartilage.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can enhance the reparative anabolic activity within articular cartilage.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate contributes to the preservation of articular cartilage, inhibits the initiation of osteoarthritic change in articular cartilage and inhibits the progression of osteoarthritic change to symptomatic osteoarthritis.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate is an effective modifier of osteoarthritic change.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can reduce the risk for osteoarthritis.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can reduce the severity of osteoarthritis.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate contributes to the preservation of articular cartilage, inhibits the initiation of inflammation in articular cartilage and inhibits the progression of rheumatoid arthritis.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can reduce the risk for rheumatoid arthritis.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can reduce the severity of rheumatoid arthritis.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate contributes to the preservation of articular cartilage, inhibits the initiation of inflammation in articular cartilage and inhibits the progression of gout.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can reduce the risk for gout.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate can reduce the severity of gout.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate produces no cardiovascular side effects.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate produces no glucoregulatory side effects.

- Dietary supplementation with *N*-acetyl-D-glucosamine, D-glucosamine, glucosamine-HCl or D-glucosamine sulfate is safe.

Summary Conclusions

Based on my review of the reliable and credible scientific literature regarding articular cartilage biochemistry and physiology, cartilage degeneration, degenerative joint disease and osteoarthritis, I conclude that the following statements are substantiated by that literature:

- "Relief First capsules are a natural COX-2 inhibitor"

- "Relief First capsules do not have the health risks associated with other COX2 inhibitors such as Vioxx"

- "Relief First capsules are good for people of any age"

Michael J. Glade, Ph.D., F.A.C.N., C.N.S.

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