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**VIA EMAIL** [mland@oc.fda.gov](mailto:mland@oc.fda.gov)  
**AND OVERNIGHT MAIL**

Michael Landa  
Deputy Chief Counsel  
Office of Chief Counsel  
Food and Drug Administration  
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Dear Michael:

I have three important points for you and the other agency representatives to ponder in advance of our meeting on the Glucosamine/Chondroitin Sulfate claim. The first is that the agency has in the recent past relied upon clinical trial data as the basis for the allowance of a health claim for disease prevention (see the phosphatidylserine/cognitive dysfunction; phosphatidylserine/dementia claims). The second is that the agency has never before construed petitioned health claims worded in disease risk reduction or prevention terms as drug claims. The third is that the evidentiary basis from the clinical studies on glucosamine and chondroitin sulfate concerns the effects at the metabolic, biochemical, cellular and tissue levels where the chondroprotective effects are expressed both in the absence of joint disease and in the presence of either asymptomatic clinically inapparent joint disease or clinically apparent joint disease. A fair consideration of those points leads ineluctably to the conclusion that the agency has erred fundamentally by reconstruing the intended disease risk reduction claims to be drug/treatment claims.

Having a heavy burden of proof to justify speech suppression, FDA lacks any empirical evidence that the claims as worded are understood to be drug treatment claims nor does any commonsense read of the language lead to that conclusion. A fair consideration of the scientific evidence leads inevitably to the conclusion that FDA has misread the import of the science on the mistaken dogma that studies in diseased populations have no relevance to healthy populations when the evidence in this case

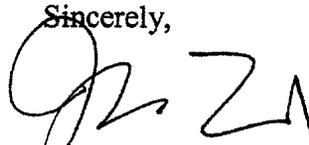
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concerning metabolic, biochemical, cellular, and tissue effects applies to both. As Dr. Glade's attached explanation reveals, the evidence is very strong indeed that glucosamine and chondroitin sulfate work as chondroprotective agents, protecting cartilage and joints from osteoarthritic lesions. That point completely escaped the agency's reviewers yet is well-established in the literature submitted.

I think our client's position very strong. If my client so directs and the agency does not change its position, I would be pleased to pursue this matter on appeal.

Sincerely,



Jonathan W. Emord

You have asked me to review FDA's correspondence of October 3, 2003, and to consider whether FDA is correct in its unsupported and unscientific assumption that the research provided to the FDA in support of the glucosamine and chondroitin sulfate health claims petition provides no credible evidence in support of the prevention claims listed in the petition. After review of both the science originally provided to the FDA and the FDA's arguments, I have found that the FDA is incorrect and misleading in its assumption.

There is no sound empirical basis for the conclusion that studies of the effects of glucosamine and chondroitin sulfate in patients with joint disease have no relevance to healthy populations. In contrast to the FDA's misinterpretation of the scientific bases of the petition, the clinical studies clearly demonstrate that the chondroprotective effects of glucosamine and chondroitin sulfate occur at the metabolic, biochemical, cellular and tissue levels where they inhibit cartilage degradation and stimulate production of new cartilage matrix. These chondroprotective effects of glucosamine and chondroitin sulfate are expressed both in the absence of joint disease and in the presence of either asymptomatic clinically inapparent joint disease or clinically apparent joint disease. Therefore, the scientific evidence confirms that the physiological effects of glucosamine and chondroitin sulfate reflect the fundamental interactions of these dietary ingredients with the cells and matrix of hyaline articular cartilage, through which the chondroprotective effects of glucosamine and chondroitin sulfate are expressed.

Contrary to the generally accepted principles of sound science, which require the evaluation of the available evidence without bias, preconceptions or conflict of interest, FDA has acted in a manner constrained by adherence to the unscientific, unfounded and widely contradicted dogma that scientific evidence obtained during the examinations of individuals with compromised physiologic or health status is not relevant to individuals who appear to enjoy uncompromised physiologic or health status, even when the appearance of uncompromised physiologic or health status may be misleading.

The observations upon which I have based these conclusions have been provided in their entirety to the FDA in the content of my previously filed report. My conclusions draw from that report and the science filed with the agency.

### Summary Conclusion

Chondroprotection is the inhibition of the progression of cartilage degradation and the stimulation of the production of new cartilage matrix. A dietary ingredient will exhibit a chondroprotective effect when it is demonstrated to inhibit the initiation of the metabolic events that produce the degenerative precursor lesions of osteoarthritis in hyaline cartilage composition or structure and to support or stimulate the biosynthesis of hyaline articular cartilage matrix components that foster or are required for normal and healthy hyaline cartilage composition or structure. As explained in my initial report and below, there can be no doubt that glucosamine and chondroitin sulfate confer chondroprotection.

As explained in my initial report and below, there can be no doubt that glucosamine and chondroitin sulfate defend articular cartilage from attack, loss, insult, injury and danger and act to keep the initial degradative precursor lesions of osteoarthritis from occurring. According to the National Institutes of Health (cited by L. Robert Lake, Letter to Jonathan W. Emord, October 3, 2003, page 2), the pathology of osteoarthritis requires the expansion of initial focal lesions in the hyaline cartilage matrix. Because the prevention or reversal of such precursor lesions by glucosamine and chondroitin sulfate provides the ultimate in risk reduction of osteoarthritic disease, there can be no doubt that glucosamine and chondroitin sulfate reduce the risk for the precursor lesions of osteoarthritis (cartilage deterioration and, if deterioration is unimpeded, joint degeneration) and, therefore, reduce the risk for osteoarthritis itself.

As explained below, "comfort" is commonly defined as freedom from pain and anxiety. Therefore, comfortable joint movement is joint movement free from pain and anxiety. A dietary ingredient that promotes, supports, or enables freedom from pain and anxiety during the use of a joint obviously reduces the risk for pain associated with the use of that joint. As explained below, glucosamine and chondroitin sulfate reduce the risk for both the precursor lesions of osteoarthritis and the pain that is produced by subsequent unimpeded progression of those lesions. In addition, because "the most compelling definition of [osteoarthritis] is one that combines the pathology of disease with pain that occurs with joint use" (Felson *et al.*, 2000, cited by L. Robert Lake, letter to Jonathan W. Emord (Oct. 3, 2003), page 2), there can be no doubt that by reducing the risk for pain that occurs with joint use, glucosamine and chondroitin sulfate reduce the risk for one of the conditions required (according to the National Institutes of Health) for the presence of osteoarthritis and, therefore, reduce the risk for osteoarthritis itself.

The scientific evidence provided to the FDA logically and strongly supports the following conclusions, which in their entirety form an undeniable basis for the further conclusion that glucosamine and chondroitin sulfate reduce the risk of cartilage deterioration, joint degeneration, osteoarthritis and osteoarthritis-related pain, tenderness, and swelling:

1. The maintenance of the biochemical, structural and functional integrity of the proteoglycan components of the extracellular matrix of articular cartilage is a required prerequisite for the preservation of healthy joint architecture and mechanical function.
2. An imbalance in cellular metabolic functions favoring catabolism within the extracellular matrix of articular cartilage compromises the biochemical, structural and functional integrity of the proteoglycan components of the extracellular matrix of articular cartilage.
3. An imbalance in cellular metabolic functions favoring catabolism within the extracellular matrix of articular cartilage produces degenerative changes in the proteoglycan composition of the matrix with net loss of healthy functioning tissue.
4. An imbalance in cellular metabolic functions favoring catabolism within the extracellular matrix of articular cartilage that compromises the structural and

functional integrity of the proteoglycan components of the extracellular matrix of articular cartilage and produces degenerative changes in the proteoglycan composition of the matrix with net loss of healthy functioning tissue results in inferior biomechanical competence of affected articular cartilage with eventual structural deformation of joint architecture.

5. 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.
6. The progression of degenerative asymptomatic subclinical osteoarthritic change is required in order for abnormalities in articular cartilage composition and structure to progress to clinically apparent and symptomatic osteoarthritis.
7. The progression of degenerative asymptomatic osteoarthritic change to clinically apparent and symptomatic osteoarthritis is not inevitable.
8. Degenerative osteoarthritic change in the absence of joint pain represents a modifiable risk factor for later development of osteoarthritis.
9. Dietary supplementation with D-glucosamine, glucosamine-HCl, glucosamine sulfate or chondroitin sulfate contributes to the preservation of articular cartilage, inhibits the initiation of degenerative osteoarthritic change in articular cartilage and inhibits the progression of degenerative osteoarthritic change to overt cartilage deterioration, inhibits the progression of overt cartilage deterioration to joint degeneration, and inhibits the progression of joint degeneration to symptomatic osteoarthritis.
10. Dietary supplementation with D-glucosamine, glucosamine-HCl, glucosamine sulfate or chondroitin sulfate is an effective modifier of degenerative osteoarthritic change and reduces the risk for osteoarthritis.
11. By reducing the risk for osteoarthritis, dietary supplementation with D-glucosamine, glucosamine-HCl, glucosamine sulfate or chondroitin sulfate reduces the risk for osteoarthritis-related pain, tenderness, and swelling.

#### Glucosamine and chondroitin sulfates are the metabolic precursors of normal cartilage.

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.<sup>1</sup> 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 collagen component of the cartilage matrix is relatively inert, but the other constituents, such as proteoglycans, 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.<sup>2</sup>

Proteoglycans are large macromolecules consisting of multiple chains of glycosaminoglycan disaccharides and oligosaccharides attached to a central protein core

that provide a framework for collagen and also bind water and cations, forming a viscous, elastic layer that lubricates and protects the cartilage tissue. 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. The glycosaminoglycans most common in human connective tissue include the disaccharides keratan sulfate, dermatan sulfate, heparin sulfate and chondroitin sulfate and the oligosaccharide, hyaluronan. They consist of amino sugars, which are repeating disaccharide units composed of a hexuronic acid (D-glucuronic acid, iduronic acid, or L-galactose) and a hexosamine (D-glucosamine or D-galactosamine).<sup>3,4</sup>

The main disaccharide units of cartilage glycosaminoglycans are formed by the (1→3) linkage of D-glucuronic acid to *N*-acetylglucosamine; disaccharide units are linked by β(1→4) galactosamine links. The D-galactosamine residues are sulfated either in position 4 (as in chondroitin-4-sulfate) or 6 (as in chondroitin-6-sulfate). 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.<sup>5-10</sup> Inadequate sulfate availability resulting in the production of undersulfated proteoglycans will reduce their electronegative charge and water carrying capacity.<sup>11,12</sup>

Glucosamine (2-amino-2-deoxyalpha-D-glucose) is an aminomonosaccharide that serves as a substrate for the biosynthesis of chondroitin sulfate, hyaluronan, and other macromolecules located in the extracellular cartilage matrix. The conversion of L-glutamine and D-fructose-6-P to L-glutamate and D-glucosamine by L-glutamine-D-fructose-6-P amidotransferase (E.C. 2.6.1.16) is the rate-limiting step in proteoglycan synthesis.<sup>13-15</sup> This reaction may be bypassed if D-glucosamine is available within the cell cytoplasm.<sup>16,17</sup> Whatever its source, D-glucosamine is phosphorylated and the resulting D-glucosamine-6-P is acetylated to *N*-acetyl-D-glucosamine, the common precursor for the biosynthesis of keratan sulfate, dermatan sulfate, chondroitin sulfate and hyaluronan.<sup>16,17</sup>

Chondroitin sulfate is a glycosaminoglycan that is polymerized into long, unbranched polysaccharide chains in which some of the constituent chondroitin moieties (composed of D-glucuronic acid and *N*-acetyl-D-glucosamine) are sulfated.<sup>18</sup> 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.<sup>19-24</sup> 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).<sup>19-21</sup> 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.<sup>19-21</sup>

Chondroitin sulfate polymers are secreted into the extracellular matrix covalently bound to proteins, forming protein-polysaccharide complexes called proteoglycans. In a

proteoglycan, about 100 chondroitin sulfate chains, each containing 50 to 60 disaccharide units of chondroitin sulfate, 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). This covalent O-linkage occurs between a terminal D-xylose or D-galactose residue that had been added to the polysaccharide chain and a serine or threonine residue on the core protein, with one chondroitin sulfate chain per 20 or so amino acid residues. The total molecular weight of an individual proteoglycan monomer is 1,500,000 to 2,500,000 daltons.<sup>5</sup>

One end of the core protein of a proteoglycan is non-covalently linked to a long polysaccharide filament of hyaluronan through a link protein; the connection is achieved by a globular region of the link protein that surrounds the terminal portion of the core protein and a stretch of 5 disaccharide units along the length of the hyaluronan chain.<sup>25,26</sup> There are two structurally related N-terminal globular domains, G1 and G2, of which only G1 (and not G2) is involved in the aggregation of proteoglycans with hyaluronan. The interglobular domain joining G1 and G2 contains proteinase-sensitive sequences which appear to be the key sites for cleavage during aggrecan turnover.<sup>5</sup> Approximately 100 core proteins are bound to an individual hyaluronan chain, at regular intervals of 300 Å, forming a unit of aggrecan, the large molecular mass proteoglycan-hyaluronan aggregate predominant within the extracellular matrix of articular cartilage.

At the molecular, biochemical, cellular and tissue levels, the basic biochemistry of hyaline articular cartilage demonstrates that glucosamine and chondroitin sulfate are the metabolic precursors of normal cartilage and are indispensable to the preservation of normal cartilage in the presence of any degradative stimuli. Therefore, glucosamine and chondroitin sulfate reduce the risk of the degradation in articular cartilage matrix quality and function that is a required precursor to osteoarthritis.

Glucosamine provides the building blocks for glycosaminoglycans, the lubricating structure in cartilage; chondroitin builds and maintains the structural framework of joints to cushion and support movement thereby reducing the risk of osteoarthritis.

I incorporate here by reference the preceding section. In addition, I add the following.

The hydrodynamic properties of the large molecular mass proteoglycan-hyaluronan 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 with little or no effect on matrix macromolecules (although sustained loads will produce slow inelastic deformation). Removal of loading

allows re-entry of water and a return to the pre-loading high-tension equilibrium condition.<sup>5,18,27-29</sup>

In cultures of chondrocytes harvested from nonosteoarthritic rat articular cartilage, IL-1 $\beta$  inhibits the expression of UDP-glucuronosyltransferase I mRNA, resulting in decreased synthesis of proteoglycans and their precursors.<sup>132,133</sup> Conversely, IL-1 $\beta$  stimulates intrachondrocytic production of the catabolism-inducing factors, NO and PGE<sub>2</sub>, resulting in increased expression of mRNA coding for the extracellular fibronectin degrading metalloproteinase enzyme, stromelysin-1.<sup>132</sup> The addition of D-glucosamine to the culture medium prevented IL-1 $\beta$ -induced inhibition of the expression of UDP-glucuronosyltransferase I mRNA<sup>132,133</sup> and of proteoglycan synthesis,<sup>132,133</sup> as well as IL-1 $\beta$ -induced activation of pro-apoptotic nuclear factor KB (NF-KB).<sup>133</sup> The addition of D-glucosamine-HCl to the culture medium of nonosteoarthritic equine articular cartilage explants in organ culture prevented IL-1 $\beta$ -induced increases in the activities of stromelysin-1, collagenase and gelatinase and bacterial lipopolysaccharide (LPS)- and IL-1 $\beta$ -induced increases in the production of NO and PGE<sub>2</sub> and the degradation of extracellular matrix proteoglycans.<sup>134-137</sup> Similarly, crystalline D-glucosamine sulfate added to the culture media of chondrocytes harvested from osteoarthritic human articular cartilage inhibited the inherent<sup>138,139</sup> and IL-1 $\beta$ -induced<sup>132,139</sup> 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.<sup>138,140</sup> By unknown but presumably similar mechanisms, dietary supplementation with D-glucosamine sulfate (50 mg/kg body weight daily) conferred to rats resistance to kaolin-and adjuvant-induced tibio-tarsal arthritis.<sup>141</sup>

Both D-glucosamine-HCl and D-glucosamine sulfate 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.<sup>14</sup> Similarly, crystalline D-glucosamine sulfate stimulated the production of proteoglycans by chondrocytes harvested from nonosteoarthritic human articular cartilage in cell culture.<sup>142</sup> 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 restored the adhesive properties of the chondrocytes,<sup>143</sup> significantly reduced extracellular collagenase activity<sup>138</sup> and significantly increased the rate of protein synthesis.<sup>138</sup> Osteoarthritic articular cartilage tissue samples harvested from rabbits that had been fed diets supplemented with D-glucosamine-HCl (20 mg/kg body weight daily) exhibited significantly accelerated rates of synthesis of new proteoglycans compared to articular cartilage tissue samples harvested from unsupplemented animals.<sup>144</sup>

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,<sup>145,146</sup> supplemental D-glucosamine stimulated the expression of IL-1 cell membrane receptor subtype II, which binds IL-1 $\beta$  with high affinity but produces an inactive receptor-ligand complex, effectively intercepting IL-1 $\beta$ -based signal transmission.<sup>133</sup> In addition, when D-glucosamine was added to the culture medium of nonosteoarthritic bovine articular cartilage explants in organ culture in concentrations that significantly inhibited IL-1 $\beta$ -induced aggrecanase cleavage of aggrecan, lactate production was unaffected and D-glucosamine was incorporated into newly-synthesized chondroitin sulfates.<sup>113</sup> D-Glucosamine-HCl also stimulated sulfate incorporation into chondroitin sulfates in the extracellular matrix of nonosteoarthritic bovine articular cartilage explants in organ culture.<sup>147</sup> In contrast, concentrations of D-glucosamine-HCl sufficiently high to compromise cell viability in nonosteoarthritic bovine articular cartilage explants in organ culture<sup>148</sup> or in nonosteoarthritic canine chondrocytes in cell culture<sup>149</sup> also significantly inhibited proteoglycan synthesis. These findings indicate that the inhibition of IL-1 $\beta$ -induced catabolism was not an artefact of D-glucosamine-induced general inhibition of chondrocyte cellular metabolism.<sup>139</sup>

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.<sup>150</sup> 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).<sup>150</sup> In addition, D-glucosamine-HCl inhibited the release of lysozymes from phagocytosing neutrophils and suppressed neutrophil chemotaxis toward zymosan-activated serum.<sup>150</sup> Furthermore, supplemental D-glucosamine inhibited the activation of T-lymphocytes and the reactivity of leukocytes without producing signs of cellular toxicity.<sup>151</sup> All of the effects of supplemental D-glucosamine provide evidence that its immunomodulatory, anabolic and anticatabolic properties result at least in part from interaction with intercellular and intracellular cytokine-based communication systems.

In cultures of chondrocytes harvested from nonosteoarthritic human knee joint articular cartilage, supplemental crystalline chondroitin sulfate polymers (Condrosulf<sup>®</sup>, Sanova Pharma, Vienna, Austria; 55% chondroitin 4-sulfate, 38% chondroitin 6-sulfate, 5% unsulfated chondroitin sulfates, 1% disulfated chondroitin sulfates, 1% non-chondroitin compounds, average molecular weight: 24,000 daltons) bind to a specific cell membrane receptor, possibly CD36, prior to transport into the chondrocyte cell cytoplasm.<sup>152</sup> When added to culture media, both Condrosulf<sup>®</sup> and a synthetic mixture of chondroitin 4-sulfate and chondroitin 6-sulfate (Structum<sup>®</sup>, Smith Kline Corp. Philadelphia, PA; undefined polydisperse mixture of chondroitin 4-sulfate and chondroitin 6-sulfate) significantly stimulated the production of proteoglycans by nonosteoarthritic human articular cartilage chondrocytes in cell culture.<sup>142, 153</sup> Paradoxically, high concentrations

(>1000 mcg/mL) of Condrosulf<sup>®</sup> in the culture medium induced concentration-dependent downregulation of the expression of mRNA coding for aggrecan.<sup>154</sup>

An undefined mix of chondroitin sulfates stimulated significant increases in the secretion of proteoglycans in nonosteoarthritic cartilage tissue<sup>155</sup> and by embryonic articular cartilage chondrocytes in cell culture.<sup>156</sup> Chondroitin 4-sulfate alone added to the culture medium stimulated significant increases in the secretion of proteoglycans and in the incorporation of sulfate into chondroitin-containing proteoglycans by embryonic articular cartilage chondrocytes in cell culture.<sup>157</sup>

A synthetic mixture of chondroitin 4-sulfate and chondroitin 6-sulfate (Structum<sup>®</sup>) prevented IL-1 $\beta$ -induced inhibition of total proteoglycan synthesis by nonosteoarthritic human articular cartilage chondrocytes in cell culture.<sup>153</sup> Similarly, undefined mixtures of chondroitin sulfates (10 mcg/mL) prevented IL-1 $\beta$ -induced inhibition of total proteoglycan synthesis<sup>158</sup> and IL-1 $\beta$ -induced stimulation of stromelysin-1 activity<sup>153</sup> in cultures of nonosteoarthritic human articular cartilage chondrocytes. Individually, both chondroitin 4-sulfate and chondroitin 6-sulfate significantly inhibited the secretion of the endopeptidase, cathepsin B, by cultured nonosteoarthritic rabbit articular chondrocytes.<sup>159</sup> However, very low concentrations of undefined mixtures of chondroitin sulfates (<1 mcg/mL) failed to significantly inhibit PGE<sub>2</sub> secretion or bacterial LPS-induced production of NO by nonosteoarthritic equine articular cartilage tissue explants in organ culture either alone or when added to significantly inhibitory concentrations of glucosamine-HCl.<sup>137</sup> In contrast, concurrent exposure of chondrocytes harvested from nonosteoarthritic articular cartilage and grown in cell culture to chondroitin sulfate (100 mcg/mL) partially inhibited the pro-apoptotic effect of NO added in amounts that when added alone significantly increased the percentage of cultures chondrocytes undergoing apoptosis.<sup>160</sup> In the same model system, although concurrent exposure was ineffective, the addition of chondroitin sulfate to the culture medium 72 hours before the addition of sodium nitroprusside (SNP) prevented SNP-induced stimulation of NO production and cellular apoptosis.<sup>161</sup>

Diets supplemented with mixed chondroitin sulfates prevented chymopapain-induced degradation of knee articular cartilage in nonosteoarthritic rabbits.<sup>162</sup> Dietary supplementation with chondroitin 6-sulfate (100 mg/kg body weight daily) significantly inhibited the destruction of articular cartilage following subsequent injection of type II collagen in Freund's adjuvant on day 14 in nonosteoarthritic mice<sup>163</sup> and significantly inhibited the depletion of proteoglycans in articular cartilage following subsequent injection of bradykinin on day 14 in nonosteoarthritic rats.<sup>164</sup>

Fragments of large chondroitin sulfate chains similar to those found in the blood after the oral ingestion of large chondroitin sulfate chains and their degradation prior to the absorption of the fragments significantly inhibited directional chemotaxis, phagocytosis and cytokine-stimulated release of lysozymes in human leukocytes in vitro.<sup>131</sup> Both

polydisperse mixtures of chondroitin sulfates<sup>165,166</sup> and Matrix<sup>®</sup> (a defined mixture of 25% chondroitin 4-sulfate and 75% chondroitin 6-sulfate<sup>167</sup>) inhibited the activity of human leukocyte elastase *in vitro*. The inhibitory activity was limited to chondroitin sulfate polymers larger in size than 2000 daltons,<sup>168,169</sup> may be limited to chondroitin 6-sulfate<sup>170</sup> and increased with the degree of sulfation of the polymers.<sup>169,171</sup> Although only an indirect indicator of events in cartilage, but consistent with an anticatabolic role for supplemental chondroitin sulfates, plasma elastase activity was significantly decreased in nonosteoarthritic rats following 8 days of dietary supplementation with a mixture of chondroitin sulfates (600 mg/kg body weight daily).<sup>5</sup> A report that *in vitro* both chondroitin 4-sulfate and chondroitin 6-sulfate compete with hyaluronan for digestion by hyaluronidase suggests an additional role for supplemental chondroitin sulfates in the direct protection of articular cartilage extracellular matrix macromolecules from the elevated degradative enzyme activities characteristic of asymptomatic subclinical cartilage degeneration.<sup>172</sup>

At the molecular, biochemical, cellular and tissue levels, the basic biochemistry of hyaline articular cartilage demonstrates that glucosamine provides the building blocks for glycosaminoglycans, the lubricating structures in cartilage, and that chondroitin sulfate builds and maintains the structural framework of joints that allows them to cushion and support movement. Therefore, glucosamine and chondroitin sulfate reduce the risk of the degradation in articular cartilage matrix quality and function that is a required precursor to osteoarthritis.

As chondroprotective agents, glucosamine and chondroitin sulfate nourish joints for comfortable movement.

I incorporate here by reference the preceding sections. In addition, as explained below in considerable detail, glucosamine and chondroitin sulfate have been found in a number of animal and human clinical studies to express the properties of chondroprotective agents that confer chondroprotection and thereby reduce the risk of cartilage deterioration, joint degeneration and osteoarthritis and osteoarthritis related joint pain, tenderness and swelling. The physiological chondroprotective effects of glucosamine and chondroitin sulfate occur at the metabolic, biochemical, cellular and tissue levels in cartilage tissue and are expressed both in the absence of joint disease and in the presence of either asymptomatic clinically inapparent joint disease or clinically apparent joint disease. The scientific evidence confirms that the physiological effects of glucosamine and chondroitin sulfate reflect the fundamental interactions of these dietary ingredients with the cells and matrix of both healthy and unhealthy hyaline articular cartilage, through which the chondroprotective effects of glucosamine and chondroitin sulfate are expressed.

In rabbits, fetal articular cartilage is softer than is adult articular cartilage because fetal articular cartilage contains a greater proportion of polysulfated chondroitin sulfates and therefore its water binding capacity is greater.<sup>30</sup> In rats, as age increases from birth to mature adulthood, the extent to which nonosteoarthritic articular cartilage extracellular

matrix chondroitins are sulfated decreases significantly.<sup>31</sup> In dogs, increasing age is accompanied by significantly decreased chondroitin sulfate and proteoglycan content of articular cartilage and reduced aggregability of the remaining proteoglycans.<sup>32</sup> Similarly, calf articular cartilage proteoglycans are larger on average than are proteoglycans in nonosteoarthritic adult bovine articular cartilage (and contain larger chondroitin sulfate polymers).<sup>33</sup> In addition to decreasing average size of matrix proteoglycans and chondroitin polymers, the ratio of chondroitin 6-sulfate to chondroitin 4-sulfate in the extracellular matrix of articular cartilage increases with increasing age.<sup>34</sup>

In humans, increasing age is accompanied by a decreasing proportion of chondroitin sulfates in the extracellular matrix of nonosteoarthritic articular cartilage<sup>35</sup> and increases in the ratio of chondroitin 6-sulfate to chondroitin 4-sulfate<sup>36,37</sup> and in the free glucosamine content of the tissue.<sup>38</sup> Furthermore, the average chondroitin sulfate content of individual articular cartilage proteoglycans decreases, impairing the ability of proteoglycans to aggregate spontaneously with hyaluronan.<sup>39</sup> In addition, the ability of proteoglycans to aggregate spontaneously with hyaluronan is decreased as a result of an increased incidence of defect in the core protein of newly-synthesized proteoglycans.<sup>40</sup> Consequently, the aggrecan content of the extracellular matrix of articular cartilage in adults is significantly lower than that in children.<sup>40</sup>

In "normal but aged" human chondrocytes (mean age of donor: 68.8 +/- 4.2 years), basal (unstimulated) synthesis of matrix-degrading stromelysin-1 and collagenase is significantly greater than in chondrocytes harvested from joints of "normal young adults" (mean age of donor: 28.6 +/- 7.1 years). Therefore, "aging" may sensitize chondrocytes to the effects of accelerators of extracellular matrix degradation and may increase the requirement of chondrocytes for exogenous substrate to support the synthesis of new and replacement matrix macromolecules.<sup>41</sup>

Osteoarthritis is a multifactorial, polygenic disorder involving mechanical, biochemical, environmental, systemic and genetic factors that contribute to imbalance between synthesis and degradation of cartilage matrix.<sup>42,43</sup> Chronic imbalance in matrix macromolecule turnover producing net loss of articular tissue is a required precursor to the development of osteoarthritis and joint pain.

There are numerous potential etiologic triggers that can initiate the progression of events culminating in tissue failure. For example, quadriceps muscle weakness significantly increases the risk for osteoarthritis in humans<sup>44</sup> and laxity in a joint may precede failure of the cartilage matrix.<sup>45</sup> 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.<sup>46</sup> A chronic imbalance of shock-absorbing and weight-bearing muscles affecting joint alignment<sup>47,48</sup> or overloading from excessive body weight<sup>49</sup> induces a mild yet chronic metabolic imbalance in the affected articular cartilage.

Whenever mechanical stress exceeds the tissue's load-bearing capacity, chondrocyte and synoviocyte secretion of the cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and nitric oxide (NO) is stimulated. These cytokines auto-stimulate chondrocyte and synoviocyte secretion of matrix metalloproteinases (collagenase, gelatinase, aggrecanase, elastase, and fibronectin-degrading stromelysin-1) and inhibit chondrocyte synthesis of cartilage-specific proteoglycans and type II collagen. The resulting imbalance between synthesis and degradation of extracellular matrix components results in a net decrease in matrix content of aggrecan, type II collagen and other matrix macromolecules.<sup>42,43,50</sup>

IL-1 $\beta$ , IL-6, TNF- $\alpha$  and nitric oxide also stimulate the clonal expansion of chondrocytes whose daughter cells may express a "fetal" differentiation pattern during early metabolic imbalance in articular cartilage<sup>51</sup> and produce inferior repair matrix prone to fibrillation and mechanical failure.<sup>42,43</sup> Spontaneous repair matrix produced in early asymptomatic subclinical osteoarthritic change exhibits a heterogeneous composition more closely resembling that of fibrous cartilage,<sup>52</sup> with inferior biomechanical competence<sup>53,54</sup> resulting in functional incompetence and perishability.<sup>55</sup> In addition, the abnormal newly-synthesized matrix may be fibronectin-deficient or may undergo accelerated hydrolysis of fibronectin by stromelysin-1, in either case disturbing chondrocyte anchorage to the extracellular matrix ("anchorage dependence") and inducing apoptosis and hypocellularity (chondrocyte survival requires attachment to substrate).<sup>42,43</sup>

In early asymptomatic subclinical osteoarthritic change in humans, reactive proliferation of extracellular articular cartilage matrix results in the production of abnormally large and more extensively sulfated chondroitin sulfate polymers and significantly decreased total glycosaminoglycan content (similar to the matrix of nonosteoarthritic human articular cartilage after partial enzymatic hydrolysis<sup>56</sup>) and significantly decreased proportion of proteoglycans of nonosteoarthritic molecular sizes.<sup>57-60</sup> Overall, there is a significantly increased proportion of nonaggregated proteoglycans, significantly decreased average size of proteoglycan aggregates (aggrecan) and incorporation of significantly smaller-than-normal-for-age chondroitin sulfate chains into newly-synthesized proteoglycans, significantly decreased total chondroitin sulfate content (and therefore decreased water binding capacity), and a significantly lower ratio of chondroitin 6-sulfate to chondroitin 4-sulfate.<sup>21,61-63</sup> Both the abnormally small proteoglycans and the abnormally large proteoglycans are unable to aggregate with hyaluronan to form aggrecan.<sup>64</sup> In addition, osteoarthritic human articular cartilage exhibits increased synthesis of more readily hydrolyzable (easily degradable) collagens.<sup>65,66</sup>

In cell culture, human articular chondrocytes harvested from osteoarthritic joint cartilage produced proteoglycans that differed from those produced by human articular chondrocytes harvested from nonosteoarthritic joint cartilage.<sup>67</sup> These proteoglycans resembled "fetal-type" proteoglycans with increased chondroitin 4-sulfate content and an increased percentage of smaller proteoglycans than is typical of the proteoglycans produced by chondrocytes harvested by nonosteoarthritic human articular cartilage.<sup>68</sup>

The synthesis of temporally inappropriate proteoglycans is accompanied by a significantly accelerated rate of degradation of older, more typical-for-age proteoglycans.<sup>69</sup>

In a rat model of the initiation of osteoarthritic change, increased mechanical stress on articular cartilage increases the ratio of chondroitin 6-sulfate to chondroitin 4-sulfate in the extracellular matrix.<sup>70</sup> Mechanical compression of articular cartilage stimulates intrachondrocytic cyclo-oxygenase activity, resulting in increased production of PGE<sub>2</sub>, an inducer of inducible NO synthase-2 (iNOS) activity; consequently, intrachondrocytic 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.<sup>71</sup> NO stimulates chondrocytic synthesis of matrix metalloproteinases,<sup>72</sup> nascent (inactive) IL-1 $\beta$ ,<sup>73-75</sup> and interleukin-1-converting enzyme (ICE).<sup>76</sup> ICE activates nascent inactive IL-1 $\beta$ .<sup>75</sup> Activated IL-1 $\beta$  inhibits chondrocytic synthesis of proteoglycans<sup>73,74</sup> and collagen<sup>73,74</sup> and stimulates chondrocytic synthesis of stromelysin-1,<sup>41</sup> collagenase<sup>41</sup> and a presumptive aggrecanase enzyme that cleaves aggrecan.<sup>77</sup> As osteoarthritic change progresses, IL-1 $\beta$  also stimulates increased NO production;<sup>78-81</sup> NO further stimulates chondrocytic synthesis of matrix metalloproteinases<sup>72</sup> and accelerates the progression of osteoarthritis through the establishment of a cooperative positive feedback cycle.<sup>82,83</sup> In addition, chondrocytes harvested from osteoarthritic human articular cartilage synthesize growth-related oncogene- $\alpha$  (GRO- $\alpha$ ) in response to IL-1 $\beta$ ; GRO- $\alpha$  stimulates degradation of fibronectin by stromelysin-1, producing anoikis (cell death resulting from loss of normal cell-substratum contact).<sup>84</sup>

Chondrocytes harvested from osteoarthritic human joints exhibit a reduced anabolic response to insulin-like growth factor-1 (IGF-1) ("IGF resistance"<sup>42,85</sup>) and may have reduced ability to transport glucose from the extracellular fluid into the cell for glycosaminoglycan synthesis.<sup>86</sup> Therefore, osteoarthritic chondrocytes may have an increased requirement for glucosamine of extracellular origin.<sup>42,87-89</sup> In addition, IGF-1 stimulates net synthesis of proteoglycans able to form aggrecan by nonosteoarthritic adult bovine articular chondrocytes in cell culture.<sup>90</sup> "IGF resistance" may contribute to the etiology of osteoarthritis by down-regulating the production of replacement aggrecan.

Oxidative stress also may impair the synthesis of matrix macromolecules by articular chondrocytes. Inhibition of chondrocyte  $\gamma$ -glutamyl-cysteine synthetase results in reduced intrachondrocytic glutathione concentration and decreased incorporation of sulfate into newly-synthesized proteoglycans and of proline into newly-synthesized collagen.<sup>91</sup>

Changes in the macromolecular composition of the extracellular matrix of articular cartilage are characteristic of clinically apparent osteoarthritis. The ratio of chondroitin 6-sulfate to chondroitin 4-sulfate in the extracellular matrix of the articular cartilage of

osteoarthritic mice is significantly greater than the ratio in the extracellular matrix of articular cartilage in age-matched nonosteoarthritic mice.<sup>92</sup> Osteoarthritic rat articular cartilage, compared to nonosteoarthritic articular cartilage, exhibits significantly decreased total proteoglycan, chondroitin 4-sulfate and chondroitin 6-sulfate contents and significantly increased stromelysin-1 (fibronectin-degrading) activity.<sup>93</sup> In addition, the percentage of apoptotic chondrocytes in the tissue is significantly increased.<sup>93</sup> 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.<sup>33</sup> Osteoarthritic equine articular cartilage contains a significantly increased proportion of unsulfated disaccharides and a significantly decreased proportion of chondroitin 6-sulfate.<sup>94</sup> The articular cartilage of *Cynomolgus* macaque monkeys with arthritis exhibits increased production of abnormal chondroitin sulfate-containing polymers.<sup>95</sup>

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.<sup>61,96,97</sup> 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.<sup>57,98,99</sup> 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.<sup>100</sup>

Pathologic changes in cartilage matrix composition and organization alter the affinity of the matrix for water and produce excessive cartilage deformation under loading.<sup>101,102</sup> When chronic, excessive tissue deformation induces adaptive structural and compositional changes that confer increased stiffness in the tissue,<sup>45</sup> increasing its vulnerability to the compressive, tensile and shear forces that occur during normal joint function.<sup>18</sup> Grossly apparent cartilage erosion does not appear until the tissue has lost considerable stiffness and is undergoing progressive mechanical failure.<sup>45</sup>

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.<sup>103</sup> 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).<sup>103</sup>

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.<sup>104</sup> Overall, the prevalence of at least mildly symptomatic osteoarthritis in at least one joint is about 30%.<sup>105</sup> Symptomatic osteoarthritis of the knee occurs in about 6% of US adults aged 30 years and older,<sup>106</sup> although radiographic changes of the femorotibial compartment occur in 5% to 15% of people aged 35 to 74 years.<sup>107</sup>

Clinical osteoarthritis (also known as degenerative joint disease) is characterized by focal loss of cartilage and hypertrophic bone spurs.<sup>103</sup> 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;<sup>108</sup> as cartilage degeneration progresses, subchondral bone density and volume increase (consistent with increased transmission of load bearing into the subchondral bone).<sup>109</sup>

The primary complaint in osteoarthritis is pain, particularly upon use of the affected joint.<sup>103</sup> 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.<sup>103</sup>

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.<sup>110</sup> 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.<sup>111</sup>

*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;<sup>112</sup> 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 30% of newly absorbed D-glucosamine sulfate was incorporated into newly synthesized proteoglycans in articular cartilage tissues.<sup>113,114</sup> 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.<sup>115</sup> 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.<sup>116,117</sup> One investigator reported that about 75% of ingested D-glucosamine sulfate was bioavailable to body tissues following hepatic first-pass extraction.<sup>117</sup>

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.<sup>118</sup>

*Chondroitin sulfate:* In dogs, rats, mice and rabbits, about 0% to 15% of an ingested mix of chondroitin sulfates was absorbed intact.<sup>42,119-124</sup> In these species, absorption favors chondroitin sulfate polymers with molecular weights <14,000 daltons.<sup>120</sup> In all species studied, some inorganic  $\text{SO}_4^{-2}$  also was absorbed following cleavage of  $\text{SO}_4^{-2}$  from the chondroitin sulfate polymers by sulfatases.<sup>42,119-124</sup>

In humans, between 0% and 15% of an oral bolus of chondroitin sulfates is absorbed intact into the blood.<sup>125-128</sup> In addition, another 10% to 20% is absorbed following hydrolysis to smaller polymers (<5000 daltons) prior to absorption.<sup>128,129</sup> However, the biological activity of these smaller polymers has been questioned.<sup>17</sup> The absorption of chondroitin sulfates probably is not nil; the consumption of either 800 mg or 3000 mg of mixed chondroitin sulfates significantly increased plasma chondroitin sulfate concentration 3 hours after ingestion<sup>130,131</sup> and the consumption of 800 mg daily for 5 days increased plasma chondroitin sulfate concentration from undetectable levels to a mean of 1.80 mcg/mL, suggesting that the systemic bioavailability of intact chondroitin sulfates in humans is about 12% of the amount ingested.<sup>131</sup>

In cultures of chondrocytes harvested from nonosteoarthritic rat articular cartilage, IL-1 $\beta$  inhibits the expression of UDP-glucuronosyltransferase I mRNA, resulting in decreased synthesis of proteoglycans and their precursors.<sup>132,133</sup> Conversely, IL-1 $\beta$  stimulates intrachondrocytic production of the catabolism-inducing factors, NO and PGE<sub>2</sub>, resulting in increased expression of mRNA coding for the extracellular fibronectin degrading metalloproteinase enzyme, stromelysin-1.<sup>132</sup> The addition of D-glucosamine to the culture medium prevented IL-1 $\beta$ -induced inhibition of the expression of UDP-glucuronosyltransferase I mRNA<sup>132,133</sup> and of proteoglycan synthesis,<sup>132,133</sup> as well as IL-1 $\beta$ -induced activation of pro-apoptotic nuclear factor KB (NF-KB).<sup>133</sup> The addition of D-glucosamine-HCl to the culture medium of nonosteoarthritic equine articular cartilage explants in organ culture prevented IL-1 $\beta$ -induced increases in the activities of

stromelysin-1, collagenase and gelatinase and bacterial lipopolysaccharide (LPS)- and IL-1 $\beta$ -induced increases in the production of NO and PGE<sub>2</sub> and the degradation of extracellular matrix proteoglycans.<sup>134-137</sup> Similarly, crystalline D-glucosamine sulfate added to the culture media of chondrocytes harvested from osteoarthritic human articular cartilage inhibited the inherent<sup>138,139</sup> and IL-1 $\beta$ -induced<sup>132,139</sup> 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.<sup>138,140</sup> By unknown but presumably similar mechanisms, dietary supplementation with D-glucosamine sulfate (50 mg/kg body weight daily) conferred to rats resistance to kaolin-and adjuvant-induced tibio-tarsal arthritis.<sup>141</sup>

Both D-glucosamine-HCl and D-glucosamine sulfate 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.<sup>14</sup> Similarly, crystalline D-glucosamine sulfate stimulated the production of proteoglycans by chondrocytes harvested from nonosteoarthritic human articular cartilage in cell culture.<sup>142</sup> 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 restored the adhesive properties of the chondrocytes,<sup>143</sup> significantly reduced extracellular collagenase activity<sup>138</sup> and significantly increased the rate of protein synthesis.<sup>138</sup> Osteoarthritic articular cartilage tissue samples harvested from rabbits that had been fed diets supplemented with D-glucosamine-HCl (20 mg/kg body weight daily) exhibited significantly accelerated rates of synthesis of new proteoglycans compared to articular cartilage tissue samples harvested from unsupplemented animals.<sup>144</sup>

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,<sup>145,146</sup> supplemental D-glucosamine stimulated the expression of IL-1 cell membrane receptor subtype II, which binds IL-1 $\beta$  with high affinity but produces an inactive receptor-ligand complex, effectively intercepting IL-1 $\beta$ -based signal transmission.<sup>133</sup> In addition, when D-glucosamine was added to the culture medium of nonosteoarthritic bovine articular cartilage explants in organ culture in concentrations that significantly inhibited IL-1 $\beta$ -induced aggrecanase cleavage of aggrecan, lactate production was unaffected and D-glucosamine was incorporated into newly-synthesized chondroitin sulfates.<sup>113</sup> D-Glucosamine-HCl also stimulated sulfate incorporation into chondroitin sulfates in the extracellular matrix of nonosteoarthritic bovine articular cartilage explants in organ culture.<sup>147</sup> In contrast, concentrations of D-glucosamine-HCl sufficiently high to compromise cell viability in nonosteoarthritic bovine articular cartilage explants in organ culture<sup>148</sup> or in nonosteoarthritic canine chondrocytes in cell culture<sup>149</sup> also significantly inhibited proteoglycan synthesis. These findings indicate

that the inhibition of IL-1 $\beta$ -induced catabolism was not an artefact of D-glucosamine-induced general inhibition of chondrocyte cellular metabolism.<sup>139</sup>

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.<sup>150</sup> 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).<sup>150</sup> In addition, D-glucosamine-HCl inhibited the release of lysozymes from phagocytosing neutrophils and suppressed neutrophil chemotaxis toward zymosan-activated serum.<sup>150</sup> Furthermore, supplemental D-glucosamine inhibited the activation of T-lymphocytes and the reactivity of leukocytes without producing signs of cellular toxicity.<sup>151</sup> All of the effects of supplemental D-glucosamine provide evidence that its immunomodulatory, anabolic and anticatabolic properties result at least in part from interaction with intercellular and intracellular cytokine-based communication systems.

In cultures of chondrocytes harvested from nonosteoarthritic human knee joint articular cartilage, supplemental crystalline chondroitin sulfate polymers (Condrosulf<sup>®</sup>, Sanova Pharma, Vienna, Austria; 55% chondroitin 4-sulfate, 38% chondroitin 6-sulfate, 5% unsulfated chondroitin sulfates, 1% disulfated chondroitin sulfates, 1% non-chondroitin compounds, average molecular weight: 24,000 daltons) bind to a specific cell membrane receptor, possibly CD36, prior to transport into the chondrocyte cell cytoplasm.<sup>152</sup> When added to culture media, both Condrosulf<sup>®</sup> and a synthetic mixture of chondroitin 4-sulfate and chondroitin 6-sulfate (Structum<sup>®</sup>, Smith Kline Corp. Philadelphia, PA; undefined polydisperse mixture of chondroitin 4-sulfate and chondroitin 6-sulfate) significantly stimulated the production of proteoglycans by nonosteoarthritic human articular cartilage chondrocytes in cell culture.<sup>142, 153</sup> Paradoxically, high concentrations (>1000 mcg/mL) of Condrosulf<sup>®</sup> in the culture medium induced concentration-dependent downregulation of the expression of mRNA coding for aggrecan.<sup>154</sup>

An undefined mix of chondroitin sulfates stimulated significant increases in the secretion of proteoglycans in nonosteoarthritic cartilage tissue<sup>155</sup> and by embryonic articular cartilage chondrocytes in cell culture.<sup>156</sup> Chondroitin 4-sulfate alone added to the culture medium stimulated significant increases in the secretion of proteoglycans and in the incorporation of sulfate into chondroitin-containing proteoglycans by embryonic articular cartilage chondrocytes in cell culture.<sup>157</sup>

A synthetic mixture of chondroitin 4-sulfate and chondroitin 6-sulfate (Structum<sup>®</sup>) prevented IL-1 $\beta$ -induced inhibition of total proteoglycan synthesis by nonosteoarthritic human articular cartilage chondrocytes in cell culture.<sup>153</sup> Similarly, undefined mixtures of chondroitin sulfates (10 mcg/mL) prevented IL-1 $\beta$ -induced inhibition of total proteoglycan synthesis<sup>158</sup> and IL-1 $\beta$ -induced stimulation of stromelysin-1 activity<sup>153</sup> in

cultures of nonosteoarthritic human articular cartilage chondrocytes. Individually, both chondroitin 4-sulfate and chondroitin 6-sulfate significantly inhibited the secretion of the endopeptidase, cathepsin B, by cultured nonosteoarthritic rabbit articular chondrocytes.<sup>159</sup> However, very low concentrations of undefined mixtures of chondroitin sulfates (<1 mcg/mL) failed to significantly inhibit PGE<sub>2</sub> secretion or bacterial LPS-induced production of NO by nonosteoarthritic equine articular cartilage tissue explants in organ culture either alone or when added to significantly inhibitory concentrations of glucosamine-HCl.<sup>137</sup> In contrast, concurrent exposure of chondrocytes harvested from nonosteoarthritic articular cartilage and grown in cell culture to chondroitin sulfate (100 mcg/mL) partially inhibited the pro-apoptotic effect of NO added in amounts that when added alone significantly increased the percentage of cultures chondrocytes undergoing apoptosis.<sup>160</sup> In the same model system, although concurrent exposure was ineffective, the addition of chondroitin sulfate to the culture medium 72 hours before the addition of sodium nitroprusside (SNP) prevented SNP-induced stimulation of NO production and cellular apoptosis.<sup>161</sup>

Diets supplemented with mixed chondroitin sulfates prevented chymopapain-induced degradation of knee articular cartilage in nonosteoarthritic rabbits.<sup>162</sup> Dietary supplementation with chondroitin 6-sulfate (100 mg/kg body weight daily) significantly inhibited the destruction of articular cartilage following subsequent injection of type II collagen in Freund's adjuvant on day 14 in nonosteoarthritic mice<sup>163</sup> and significantly inhibited the depletion of proteoglycans in articular cartilage following subsequent injection of bradykinin on day 14 in nonosteoarthritic rats.<sup>164</sup>

Fragments of large chondroitin sulfate chains similar to those found in the blood after the oral ingestion of large chondroitin sulfate chains and their degradation prior to the absorption of the fragments significantly inhibited directional chemotaxis, phagocytosis and cytokine-stimulated release of lysozymes in human leukocytes *in vitro*.<sup>131</sup> Both polydisperse mixtures of chondroitin sulfates<sup>165,166</sup> and Matrix<sup>®</sup> (a defined mixture of 25% chondroitin 4-sulfate and 75% chondroitin 6-sulfate<sup>167</sup>) inhibited the activity of human leukocyte elastase *in vitro*. The inhibitory activity was limited to chondroitin sulfate polymers larger in size than 2000 daltons,<sup>168,169</sup> may be limited to chondroitin 6-sulfate<sup>170</sup> and increased with the degree of sulfation of the polymers.<sup>169,171</sup> Although only an indirect indicator of events in cartilage, but consistent with an anticatabolic role for supplemental chondroitin sulfates, plasma elastase activity was significantly decreased in nonosteoarthritic rats following 8 days of dietary supplementation with a mixture of chondroitin sulfates (600 mg/kg body weight daily).<sup>5</sup> A report that *in vitro* both chondroitin 4-sulfate and chondroitin 6-sulfate compete with hyaluronan for digestion by hyaluronidase suggests an additional role for supplemental chondroitin sulfates in the direct protection of articular cartilage extracellular matrix macromolecules from the elevated degradative enzyme activities characteristic of asymptomatic subclinical cartilage degeneration.<sup>172</sup>

Chondroprotection is the inhibition of the progression of cartilage degradation and the stimulation of the production of new cartilage matrix.<sup>3,16,137,219</sup> A dietary ingredient will exhibit a chondroprotective effect when it is demonstrated to inhibit the initiation of the metabolic events that produce the degenerative precursor lesions of osteoarthritis in hyaline cartilage composition or structure.<sup>5,12,18,78,119,150,158,160,172,201</sup> “By definition, a chondroprotective agent is a substance which is capable of increasing chondrocyte anabolic activity, while simultaneously suppressing the degradative action of mediators (cytokines, prostaglandins, proteinases) on cartilage.”<sup>142</sup> As demonstrated above, there can be no doubt that glucosamine and chondroitin sulfate are chondroprotective agents that confer chondroprotection.

According to *The Random House Dictionary of the English Language, College Edition* (Random House, NY, 1968), to “protect” is “to defend or guard from attack, loss, insult, etc.; cover or shield from injury or danger.” To “prevent” is “to keep from occurring.” As demonstrated above, there can be no doubt that glucosamine and chondroitin sulfate defend articular cartilage from attack, loss, insult, injury and danger and act to keep the initial degradative precursor lesions of osteoarthritis from occurring. According to the National Institutes of Health,<sup>45</sup> (cited by L. Robert Lake, letter to Jonathan W. Emord (undated), page 2), the pathology of osteoarthritis requires the expansion of initial focal lesions in the hyaline cartilage matrix. Because the prevention or reversal of such precursor lesions by glucosamine and chondroitin sulfate provides the ultimate in risk reduction of osteoarthritic disease, as defined by the National Institutes of Health,<sup>45</sup> (cited by L. Robert Lake, letter to Jonathan W. Emord (undated), page 2), there can be no doubt that glucosamine and chondroitin sulfate reduce the risk for the precursor lesions of osteoarthritis (cartilage deterioration and, if deterioration is unimpeded, joint degeneration) and, therefore, reduce the risk for osteoarthritis itself.

According to *The Random House Dictionary of the English Language, College Edition* (Random House, NY, 1968), “comfort” is “freedom from pain and anxiety.” Therefore, comfortable joint movement is joint movement free from pain and anxiety. A dietary ingredient that promotes, supports or enables “freedom from pain and anxiety” during the use of a joint obviously reduces the risk for pain associated with the use of that joint. As demonstrated above, glucosamine and chondroitin sulfate reduce the risk for both the precursor lesions of osteoarthritis and the pain that is produced by subsequent unimpeded progression of those lesions. In addition, because “the most compelling definition of [osteoarthritis] is one that combines the pathology of disease with pain that occurs with joint use” (Felson *et al.*, 2000,<sup>45</sup> cited by L. Robert Lake, letter to Jonathan W. Emord (undated), page 2), there can be no doubt that by reducing the risk for pain that occurs with joint use, glucosamine and chondroitin sulfate reduce the risk for one of the conditions required (according to the National Institutes of Health) for the presence of osteoarthritis and, therefore, reduce the risk for osteoarthritis itself.

Human clinical studies confirm that glucosamine and chondroitin sulfate reduce the risk for the precursor lesions of osteoarthritis and for the pain produced by any subsequent unimpeded progression of those lesions.

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. 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 months was no better than the consumption of placebo in relieving joint pain in individuals with mild to severe femorotibial osteoarthritis.

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<sup>®</sup>; 1500 mg/day) for 1 month has produced significantly greater reductions in articular pain, tenderness, swelling and restriction of movement.<sup>176,177</sup> In another study, short-term dietary supplementation with Dona<sup>®</sup> (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.<sup>178</sup> 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<sup>®</sup> (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.<sup>179</sup>

In long-term randomized double-blind placebo-controlled clinical studies, compared to the effects of placebo, 3 years of dietary supplementation with Dona<sup>®</sup> (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<sup>180</sup>), 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.<sup>180,181</sup> 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<sup>®</sup> (1500 mg/day) for 13 to 99 days.<sup>182</sup> 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<sup>®</sup> (1500 mg/day for 4 days, then 750 mg/day for 90 to 120 additional days.<sup>183</sup> 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<sup>®</sup> (1500 mg/day) or ibuprofen (1200 mg/day) for 4 weeks.<sup>184,185</sup> In both studies, both groups experienced similar significant decreases in the Lequesne index of functional impairment<sup>184</sup> and in pain at rest, pain during movement, pain under loading and joint swelling<sup>185</sup> (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<sup>®</sup> (1500 mg/day) or ibuprofen (1200 mg/day) for 8 weeks.<sup>186</sup> In this study, Dona<sup>®</sup> 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<sup>™</sup>; 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).<sup>187</sup> 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 investigator concluded that the randomized double-blind placebo-controlled studies of adequate quality to include in the analysis demonstrated that dietary supplementation with D-glucosamine sulfate 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”).<sup>188</sup> 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,<sup>189</sup> that dietary supplementation with D-glucosamine sulfate is “probably effective in osteoarthritis in reducing pain and in improving joint function”<sup>107</sup> 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.<sup>190</sup>

When only “high quality” studies were considered by another investigator, it was concluded that dietary supplementation with D-glucosamine sulfate by individuals with osteoarthritis produced an approximately 50% reduction in pain with a similar improvement in function (a “large” effect consistently greater than that of placebo).<sup>191</sup> 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.<sup>191</sup>

In randomized, double-blind, placebo-controlled studies of subjects with femorotibial osteoarthritis ranging from mild to severe, compared to the effects of placebo, dietary supplementation with Condrosulf<sup>®</sup> (Sanova Pharma, Vienna, Austria; 55% chondroitin 4-sulfate, 38% chondroitin 6-sulfate, 5% unsulfated chondroitin sulfates, 1% disulfated chondroitin sulfates, 1% non-chondroitin compounds, average molecular weight: 24,000 daltons; 800 mg/day for 6 months,<sup>192</sup> 1 year<sup>193</sup> or 2 years<sup>194</sup> or 1200 mg/day for 3 months<sup>195</sup> or 6 months<sup>196</sup>) consistently produced significantly greater decreases in the Lequesne Index of functional impairment and in the severity of spontaneous joint pain assessed using a visual analog scale. After 1 year, subjects consuming 800 mg/day also exhibited significantly smaller increases in serum concentrations of osteocalcin (a biomarker of new bone formation) and keratan sulfate (a biomarker of proteoglycan degradation) and urinary excretions of pyridinoline and deoxypyridinoline (biomarkers of collagen degradation), although overall mobility and the mean rate of narrowing of the internal femorotibial space were not affected by dietary supplementation with chondroitin sulfates.<sup>193</sup> However, subjects consuming 800 mg/day for 2 years exhibited significantly smaller decreases in the mean internal femorotibial space.<sup>194</sup> In none of these studies did dietary supplementation with chondroitin sulfates produce a significant increase in the incidence or severity of side effects.<sup>192-196</sup>

In a randomized, double-blind, placebo-controlled study of subjects with osteoarthritis of the hip ranging from mild to severe, daily supplementation with Condrosulf<sup>®</sup> (1200 mg

for 24 weeks) produced significantly greater decreases in the Lequesne Index of functional impairment, the severity of spontaneous joint pain assessed using a visual analog scale and the voluntary use of nonsteroidal anti-inflammatory drugs (NSAID's) for rescue from pain, although in this short-term study the mean rate of narrowing of the internal femorotibial space was not affected by dietary supplementation with chondroitin sulfates.<sup>197</sup> In a randomized, double-blind, placebo-controlled study of subjects with osteoarthritis of the interphalangeal joints, daily supplementation with Condrosulf<sup>®</sup> (1200 mg for 3 years) produced a significantly greater decrease in the number of subjects whose osteoarthritis had progressed.<sup>198</sup> However, there was no effect on the progression of osteoarthritis of the metacarpal joints. Even after as long as 3 years of daily supplementation there were no significant differences in the incidence or severity of side effects or adverse reactions.<sup>198</sup>

In three short-term open-label studies, dietary supplementation with Condrosulf<sup>®</sup> (400 mg/day,<sup>199</sup> 800 mg/day<sup>200</sup> or 1200mg/day<sup>201</sup> for 3 months) significantly reduced the severity of spontaneous joint pain assessed using a visual analog scale and the voluntary use of nonsteroidal anti-inflammatory drugs (NSAID's) for rescue from pain in subjects with osteoarthritis of the interphalangeal, femorotibial or hip joints. One study reported 97% subject compliance with supplementation<sup>200</sup> and no study reported clinically significant side effects.

In a randomized, double-blind, placebo-controlled study of subjects with early mild femorotibial osteoarthritis, 2 years of dietary supplementation with Matrix<sup>®</sup> (25% chondroitin 4-sulfate and 75% chondroitin 6-sulfate; IRBI S.p.A., Rome, Italy; 800 mg/day) produced significantly greater decreases in the severity of spontaneous joint pain assessed using a visual analog scale and the voluntary use of nonsteroidal anti-inflammatory drugs (NSAID's) for rescue from pain.<sup>202</sup> In another randomized, double-blind, placebo-controlled study of subjects with early mild femorotibial osteoarthritis, 330 days of dietary supplementation with Matrix<sup>®</sup> (200 mg/day) produced significantly greater decreases in the severity of spontaneous joint pain assessed using a visual analog scale, pain on passive movement, pain on active movement, and pain in the evening; significantly greater increases in joint mobility and ambulation; and a significantly smaller decrease in mean articular cartilage thickness (measured echographically).<sup>203</sup> In neither trial were the incidence or severity of side effects or adverse reactions significantly different among the subjects consuming Matrix<sup>®</sup> or placebo. Similarly, in an open label pilot study, dietary supplementation with Matrix<sup>®</sup> (1200 mg/day for 2 months) significantly reduced the severity of spontaneous joint pain assessed using a visual analog scale without producing side effects.<sup>204</sup>

Subjects with femorotibial osteoarthritis ranging from mild to severe have participated in three studies during which one group of subjects received supplemental Condrosulf<sup>®</sup> (800 mg/day) for 3 months, placebo for 3 months, Condrosulf<sup>®</sup> (800 mg/day) for 3 months and placebo for 3 months while a second group of subjects consumed only placebo for 12 months.<sup>205-207</sup> In all 3 studies, after 12 months, despite having consumed

only placebo for the 3 months prior, subjects previously supplemented with Condrosulf<sup>®</sup> exhibited significantly greater decreases in the Lequesne Index of functional impairment, the severity of spontaneous joint pain assessed using a visual analog scale and the mean rate of narrowing of the internal femorotibial space. In one study, the supplemented subjects also exhibited significantly smaller decreases in femorotibial joint articular surface area, femorotibial joint space volume and femoral articular cartilage thickness.<sup>206</sup> In addition, between months 9 and 12 in this study (when both groups consumed placebo), unlike the subjects consuming placebo for 12 months, the subjects previously consuming Condrosulf<sup>®</sup> did not experience rebound increase in pain.<sup>206</sup> In another study, after 12 months, despite having consumed only placebo for the 3 months prior, subjects previously supplemented with Condrosulf<sup>®</sup> exhibited significantly smaller increases in the serum concentration of cartilage oligomeric matrix protein (COMP) (a biomarker of synovial and cartilage inflammation), serum concentration of keratan sulfate (a biomarker of proteoglycan degradation), and urinary excretion of pyridinoline and deoxypyridinoline (biomarkers of collagen degradation), confirming the anticatabolic properties of supplemental chondroitin sulfates.<sup>205</sup> In contrast, serum concentrations of osteocalcin were not significantly affected, suggesting that supplemental chondroitin sulfates do not actively influence bone metabolism or turnover.<sup>205</sup> In none of the 3 trials were the incidence or severity of side effects or adverse reactions significantly different among the subjects consuming Condrosulf<sup>®</sup> or placebo.<sup>205-207</sup>

The effectiveness of dietary supplementation with Condrosulf<sup>®</sup> in reducing pain has been compared directly to the effectiveness of NSAID's. Subjects with femorotibial osteoarthritis ranging from mild to severe were supplemented with either Condrosulf<sup>®</sup> (1200 mg/day) or diclofenac sodium (150 mg/day) for 3 months, after which both groups of subjects were supplemented with placebo for another 3 months.<sup>208</sup> After 3 months of active supplementation, both groups of subjects exhibited similar significant decreases (compared to baseline) in the Lequesne Index of functional impairment, the severity of pain at rest assessed using a visual analog scale and the severity of pain on loading assessed using a visual analog scale. However, after 3 subsequent months of placebo, pain increased significantly in subjects previously supplemented with diclofenac sodium but did not increase in subjects previously supplemented with Condrosulf<sup>®</sup>.

In two preliminary open-label studies, the diets of subjects with osteoarthritis of the femorotibial or hip joints were supplemented with 1000 mg/day to 1500 mg/day of Structum<sup>®</sup> (Smith Kline Corp. Philadelphia, PA; undefined polydisperse mixture of chondroitin 4-sulfate and chondroitin 6-sulfate) for up to 4 months.<sup>209,210</sup> Supplementation was reported to have produced significant decreases in pain, with up to 85% of subjects reporting reduced pain and enhanced mobility.<sup>210</sup> When the diets of similar subjects were supplemented with 1000 mg/day of Structum<sup>®</sup> for 6 months, pain at rest disappeared completely in 57% of subjects with femorotibial osteoarthritis and in 46% of subjects with osteoarthritis of the hip and pain with movement disappeared

completely in 17% of subjects with femorotibial osteoarthritis and in 13% of subjects with osteoarthritis of the hip.<sup>211</sup> During the 6 months of this open-label study, joint function improved significantly while voluntary consumption of NSAID's for rescue from pain decreased significantly. In a similar 3-month placebo-controlled study, 1000 mg/day of Structum<sup>®</sup> produced significantly greater decreases in the Lequesne Index of functional impairment, the severity of pain at rest assessed using a visual analog scale and voluntary consumption of NSAID's for rescue from pain.<sup>212</sup> After 3 subsequent months of discontinuation of supplementation, no significant return of pain occurred among the previously-supplemented subjects. There were no clinically significant side effects in any reported study of dietary supplementation with Structum<sup>®</sup>.<sup>209-212</sup>

In a randomized placebo-controlled study of subjects with femorotibial osteoarthritis, dietary supplementation with mixed chondroitin sulfates (1000 mg/day; unknown source) for 3 months produced significantly greater decreases in the Lequesne Index of functional impairment and the severity of pain at rest assessed using a visual analog scale.<sup>213</sup> No significant differences occurred in the severity of pain with activity assessed by use of a visual analog scale or in the incidence or severity of side effects. Among the previously-supplemented subjects, pain had not returned three months after discontinuation of supplementation. In a randomized placebo-controlled study of subjects with severe osteoarthritis of the proximal interphalangeal joints, dietary supplementation with mixed chondroitin sulfates (800 mg/day; unknown source) for 2 years produced significantly greater decreases in the depth of erosions of the femoral articular surface and in the number of painful joints per subject.<sup>214</sup>

Subjects with severe osteoarthritis of the proximal interphalangeal joints received dietary supplementation with Chondral<sup>®</sup> (a polydisperse mixture of chondroitin sulfates; Societa Prodotti Antibiotici, Milan, Italy; 800 mg/day) either with or without naproxen (500 mg/day).<sup>215</sup> After 2 years, the combination of Chondral<sup>®</sup> and naproxen produced significantly slower progression of cartilage erosions, but the progression of clinical osteoarthritis was not arrested.

Several groups of investigators have applied the techniques of meta-analysis to evaluate dietary supplementation with chondroitin sulfates. One group concluded that 7 randomized double-blind placebo-controlled studies were of adequate quality to include in their analysis.<sup>216</sup> These studies demonstrated that when consumed at 1200 mg daily for at least 120 days, dietary supplementation with chondroitin sulfates produced significantly greater reductions in the Lequesne Index of functional impairment and in the severity of pain assessed using a visual analog scale than did placebo (the effect size was "large").<sup>216</sup> In addition, 65% of subjects consuming chondroitin sulfates will be expected to benefit more than if they were consuming placebo and, in general, adverse effects were more frequent when placebo was consumed than when chondroitin sulfates were consumed.<sup>216</sup> Other investigators concluded that dietary supplementation with chondroitin sulfates by individuals with osteoarthritis consistently produced significant decreases in joint pain and significant increases in joint function of small-to-moderate

magnitude<sup>189</sup> and that dietary supplementation with chondroitin sulfates is “probably effective in osteoarthritis in reducing pain and in improving joint function.”<sup>107</sup>

When only “high quality” studies were considered by another investigator, it was concluded that dietary supplementation with chondroitin sulfates by individuals with osteoarthritis produced an approximately 50% reduction in pain with a similar improvement in function (a “large” effect consistently greater than that of placebo).<sup>191</sup> However, it was noted that the quality of most published studies concerning dietary supplementation with chondroitin sulfates by individuals with osteoarthritis has been generally poor and that the magnitude of the reported effects of dietary supplementation with chondroitin sulfates are likely to be inflated by weaknesses in the study designs and analysis. Nonetheless, it was concluded that, despite their flaws, the available published studies demonstrate a significant degree of efficacy for dietary supplementation with chondroitin sulfates.<sup>191</sup>

The application of a pharmacokinetic-pharmacodynamic model of intake-dependent effects resulted in the estimation that dietary supplementation with chondroitin sulfates (especially Condrosulf<sup>®</sup>, 1200 mg/day) “can reduce baseline pain and algofunctional indices by over 80%”; it was estimated that about half of this benefit can be experienced in about 35 days of supplementation.<sup>217</sup>

In an open-label trial, subjects with femorotibial osteoarthritis consumed either D-glucosamine sulfate (unknown source; 1500 mg/day) or mixed chondroitin sulfates (unknown source; 675 mg/day).<sup>218</sup> After 3 months there were no significant differences between the two groups of subjects; 72% of all subjects self-assessed their improvement as “good” without side effects. The extent of improvement in pain during moderate exercise following supplementation was inversely proportional to the severity of pain during moderate exercise prior to the initiation of supplementation (subjects with the most severe pain responded the least to either dietary supplement).

In a rabbit surgical model of osteoarthritis, following surgery five groups of rabbits were fed either a control diet or the control diet supplemented with either D-glucosamine HCl, mixed chondroitin sulfates, manganese ascorbate or Cosamin<sup>®</sup> for 16 weeks.<sup>219</sup> Upon sacrifice, the rabbits fed either the control diet or the control diet supplemented with either D-glucosamine HCl, mixed chondroitin sulfates, or manganese ascorbate exhibited no significant differences in the area of articular cartilage surface lesions, the percentage of animals exhibiting severe lesions, the rates of synthesis or degradation of articular cartilage proteoglycans or the magnitude of IL-1 $\beta$ -induced collagenase activity. In contrast, dietary supplementation with Cosamin<sup>®</sup> produced significantly greater decreases in the area of articular cartilage surface lesions, the percentage of animals exhibiting severe lesions and the magnitude of IL-1 $\beta$ -induced collagenase activity. In this model, Cosamin<sup>®</sup> had no effect on the rates of synthesis or degradation of articular cartilage proteoglycans.

In a randomized double-blind placebo-controlled clinical trial, subjects with mild to moderate femorotibial osteoarthritis supplemented their diets with either a combination of D-glucosamine-HCl (1000 mg/day), mixed chondroitin sulfates (800 mg/day) and manganese ascorbate (152 mg/day) (2 tablets of Cosamin<sup>®</sup>; Nutramax Laboratories, Inc.) or placebo.<sup>220</sup> After 6 months, dietary supplementation with Cosamin<sup>®</sup> was associated with significantly greater decreases in the Lequesne index of functional impairment, although there was no difference in WOMAC pain scores or in subject self-assessment between subjects supplemented with Cosamin<sup>®</sup> and those supplemented with placebo. In addition, subjects with severe femorotibial osteoarthritis experienced little improvement. There also was no difference in the incidence or severity of side effects. In another randomized double-blind placebo-controlled clinical trial, subjects with mild to moderate femorotibial osteoarthritis or osteoarthritis of the lower back supplemented their diets with either a combination of D-glucosamine-HCl (1500 mg/day), mixed chondroitin sulfates (1200 mg/day) and manganese ascorbate (228 mg/day) (3 tablets of Cosamin<sup>®</sup>; Nutramax Laboratories, Inc.) or placebo.<sup>221</sup> After 16 weeks, Cosamin<sup>®</sup>-supplemented subjects with femorotibial osteoarthritis exhibited significantly greater decreases in summary disease score, subject self-assessment of pain, severity of pain assessed using a visual analog scale, but there was no difference in maximum running times. Subjects with osteoarthritis of the lower back exhibited no response to the dietary supplement. There were no intergroup differences in the incidence or severity of side effects.

In a randomized double-blind placebo-controlled trial, dietary supplementation with D-glucosamine-HCl (1500 mg/day) plus mixed chondroitin sulfates (1200 mg/day) was compared with placebo in subjects with painful osteoarthritis of the temporomandibular joint.<sup>222</sup> After 12 weeks, subjects supplemented with D-glucosamine-HCl plus mixed chondroitin sulfates exhibited significantly greater decreases in temporomandibular joint tenderness and sounds and in voluntary consumption of pain relieving medications without the production of side effects. In an uncontrolled study of subjects with chronic temporomandibular joint osteoarthritis, 80% of subjects reported self-perceived decreases in temporomandibular joint sounds during dietary supplementation with a combination of D-glucosamine-HCl (1600 mg/day), mixed chondroitin 4- and 6-sulfates (1200 mg/day) and calcium ascorbate (1000 mg/day) (NOW Foods, Glendale Heights, IL).<sup>223</sup>

The reliable and credible scientific literature indicates that daily dietary supplementation with 1500 mg of D-glucosamine sulfate is effective in reducing the risk of osteoarthritis.

When combined with dietary supplementation with chondroitin sulfates (at least 800 mg/day), daily dietary supplementation with 1000 mg of D-glucosamine-HCl is effective in reducing the risk of osteoarthritis.

The reliable and credible scientific literature indicates that daily dietary supplementation with 1200 mg of chondroitin sulfate (containing chondroitin 4-sulfate and chondroitin 6-sulfate in approximately equal proportions), whether consumed alone or in combination with supplemental D-glucosamine sulfate or D-glucosamine-HCl is effective in reducing the risk of osteoarthritis.

As demonstrated above, glucosamine and chondroitin sulfate reduce or halt the progression of the lesions that produce loss of flexibility and mobility, with the direct or indirect consequence of a reduction in the pain that may be associated with such loss. Because sufficient prevention of lesion progression to prevent pain includes the prevention or reversal of precursor lesions, there can be no doubt that glucosamine and chondroitin sulfate reduce the risk for both the precursor lesions of osteoarthritis and the pain that is produced by subsequent unimpeded progression of those lesions.

### **Conclusions**

- The maintenance of the biochemical, structural and functional integrity of the proteoglycan components of the extracellular matrix of articular cartilage is a required prerequisite for the preservation of healthy joint architecture and mechanical function.
- An imbalance in cellular metabolic functions favoring catabolism within the extracellular matrix of articular cartilage compromises the biochemical, structural and functional integrity of the proteoglycan components of the extracellular matrix of articular cartilage.
- An imbalance in cellular metabolic functions favoring catabolism within the extracellular matrix of articular cartilage produces degenerative changes in the proteoglycan composition of the matrix with net loss of healthy functioning tissue.
- An imbalance in cellular metabolic functions favoring catabolism within the extracellular matrix of articular cartilage that compromises the structural and functional integrity of the proteoglycan components of the extracellular matrix of articular cartilage and produces degenerative changes in the proteoglycan composition of the matrix with net loss of healthy functioning tissue results in inferior biomechanical competence of affected articular cartilage with eventual 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 progression of degenerative asymptomatic subclinical osteoarthritic change is required in order for abnormalities in articular cartilage composition and structure to progress to clinically apparent and symptomatic osteoarthritis.
- The progression of degenerative asymptomatic osteoarthritic change to clinically apparent and symptomatic osteoarthritis is not inevitable.

- Degenerative osteoarthritic change in the absence of joint pain represents a modifiable risk factor for later development of osteoarthritis.
- Dietary supplementation with D-glucosamine, glucosamine-HCl, glucosamine sulfate or chondroitin sulfate contributes to the preservation of articular cartilage, inhibits the initiation of degenerative osteoarthritic change in articular cartilage and inhibits the progression of degenerative osteoarthritic change to overt cartilage deterioration, inhibits the progression of overt cartilage deterioration to joint degeneration, and inhibits the progression of joint degeneration to symptomatic osteoarthritis.
- Dietary supplementation with D-glucosamine, glucosamine-HCl, glucosamine sulfate or chondroitin sulfate is an effective modifier of degenerative osteoarthritic change and reduces the risk for osteoarthritis.
- By reducing the risk for osteoarthritis, dietary supplementation with D-glucosamine, glucosamine-HCl, glucosamine sulfate or chondroitin sulfate reduces the risk for osteoarthritis-related pain, tenderness, and swelling.

#### **Restatement of Report Conclusions**

In conclusion, having studied carefully and in detail both the science originally provided to the FDA and FDA's October 3, 2003 letter, I find no sound scientific or logical basis for the conclusion that the following risk reduction claims are not supported by the scientific evidence provided to the FDA. To the contrary, I find significant scientific agreement in support of these health claims:

- Glucosamine may reduce the risk of osteoarthritis.
- Chondroitin sulfate may reduce the risk of osteoarthritis.
- Glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis.
- Glucosamine may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Chondroitin sulfate may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Glucosamine and chondroitin sulfate may reduce the risk of osteoarthritis-related joint pain, tenderness and swelling.
- Glucosamine may reduce the risk of joint degeneration.
- Chondroitin sulfate may reduce the risk of joint degeneration.

- Glucosamine and chondroitin sulfate may reduce the risk of joint degeneration.
- Glucosamine may reduce the risk of cartilage deterioration.
- Chondroitin sulfate may reduce the risk of cartilage deterioration.
- Glucosamine and chondroitin sulfate may reduce the risk of cartilage deterioration.

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