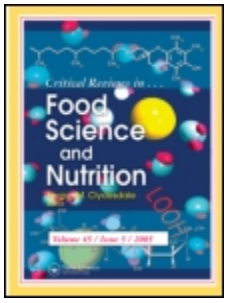

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Nutraceutical Therapies for Degenerative Joint Diseases: A Critical Review

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There is growing recognition of the importance of nutritional factors in the maintenance of bone and joint health, and that nutritional imbalance combined with endocrine abnormalities may be involved in the pathogenesis of osteoarthritis (OA) and osteochondritis dissecans (OCD). Despite this, dietary programs have played a secondary role in the management of these connective tissue disorders. Articular cartilage is critically dependent upon the regular provision of nutrients (glucose and amino acids), vitamins (particularly vitamin C), and essential trace elements (zinc, magnesium, and copper). Therefore, dietary supplementation programs and nutraceuticals used in conjunction with non-steroidal, anti-inflammatory drugs (NSAIDs) may offer significant benefits to patients with joint disorders, such as OA and OCD. This article examines the available clinical evidence for the efficacy of nutraceuticals, antioxidant vitamin C, polyphenols, essential fatty acids, and mineral cofactors in the treatment of OA and related joint disorders in humans and veterinary species. This article also attempts to clarify the current state of knowledge. It also highlights the need for additional targeted research to elucidate the changes in nutritional status and potential alterations to the expression of plasma membrane transport systems in synovial structures in pathophysiological states, so that current therapy and future treatments may be better focused.

Keywords articular cartilage, essential fatty acids, glucosamine sulphate, glucose transport, nutraceutical, nutrition, osteoarthritis, osteochondritis, vitamin C

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Abbreviations: CMGP, cartilage matrix glycoprotein; COX-2, cyclo-oxygenase-2; CS, chondroitin sulphate; DHA, docosahexaenoic acid; EC, (-)-epicatechin; ECG, (-)-epicatechin gallate; ECM, extracellular matrix; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate; EPA, eicosapentaenoic acid; Erk, extracellular signal-regulated kinase; GAGs, glycosaminoglycans; GlcAT-1, galactose-b(beta)-1,3-glucuronosyltransferase; GS, glucosamine sulphate; GHCl, glucosamine hydrochloride; GLM, Green Lipped Mussel; GLUT/SLC2A, glucose transporter (gene symbol SLC2A); GPI, glycosyl-phosphatidylinositol; IFN-γ, Interferon gamma; IGF-1 insulin-like growth factor 1; IL-1α, interleukin-1 alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; cJNK, c Jun-N-terminal kinase; LOX, lipoxygenase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MMP-3, matrix metalloproteinase-3; NO, Nitric Oxide; NF-κB, nuclear factor kappa B; NMR, nuclear magnetic resonance; NSAIDs, non-steroidal anti-inflammatory drugs; OA, osteoarthritis; OC, osteochondrosis; OCD, osteochondritis dissecans; PGs, proteoglycans; PGE_2, Prostaglandin E_2; PUFAs, polyunsaturated fatty acids; RA, rheumatoid arthritis; SVCT2, sodium-dependent vitamin C transporter 2; TNF-α, tumour necrosis factor alpha; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

INTRODUCTION

Nutrition may be linked to a range of degenerative and developmental disorders. Nutritional deficiencies and imbalances in humans and animals result in metabolic and systemic disturbances that may increase susceptibility to joint disease. Degenerative conditions in cartilage, such as osteoarthritis (OA), often occur coincidentally with metabolic dysfunction, nutrient imbalance, and diabetes mellitus (Rosenbloom and Silverstein, 1996; Denko and Malemud, 1999; Okma-Keulen and Hopman-Rock, 2001). Clinical and epidemiological surveys of OA patients with diabetes mellitus support the hypothesis that hyperglycaemia affects matrix macromolecules and may be related to the development of degenerative joint and bone disease (Cimmino and Cutulo, 1990). Musculoskeletal problems, such as osteochondritis dissecans (OCD), in rapidly growing domesticated species, commonly have a multifactorial etiology, including a nutrition related component. OCD results from aberrant endochondral ossification, wherein articular cartilage becomes abnormally thickened and subsequently fragments (Williams et al., 1998). Although mechanical, hormonal, and genetic influences contribute to joint disease, the involvement of nutrition in the etiology of these disorders raises the possibility that strategies adopted for preventing cartilage degradation may incorporate dietary manipulation. Nutritional management alone may be of limited consequence with regard to the pathogenesis of developmental diseases, such as OCD. However, the progression of such diseases may be influenced during growth by the provision of a diet with an appropriate nutrient profile (Richardson and Zentek, 1998). This critical article summarizes the current information on the mechanisms responsible for the uptake of nutraceuticals and micronutrients into chondrocytes in articular cartilage and evaluates the evidence for the use of nutraceuticals in the treatment of degenerative disorders of cartilage, particularly OA. This review focuses mainly on the effects of nutraceuticals, antioxidants, n-3 polyunsaturated fatty acids (PUFAs), and micronutrients on OA cartilage, and rheumatoid arthritis (RA) is rarely mentioned. Evidence of the nutritional management of RA has recently been reviewed elsewhere, and we refer the readers to an excellent article by Rennie and colleagues (2003) and a meta-analysis of previously published data by Muller and colleagues (2001).

Nutrient Uptake is Critical for Cartilage Function

Chondrocytes exist within and maintain the extracellular matrix (ECM) of the avascular, aneural environment of articular cartilage. Articular cartilage is composed of two main extracellular components, collagen and proteoglycan (Figure 1). The collagen fibrillar network, composed primarily of type II collagen, confers tensile strength to the tissue, resisting the swelling pressure exerted by the hydrated glycosaminoglycans (GAGs). As a result of these two opposing forces, cartilage is able to resist deformation by the mechanical compression forces to which it is exposed when load is applied to the joint, e.g., during walking. Free or degraded GAGs can diffuse out of the cartilage easily. However, GAGs are retained by healthy cartilage, as they are generally bound to large core proteins to produce aggregating proteoglycans (PGs), such as aggrecan. Although other ECM proteins, such as laminin and fibronectin, in turn bind to chondrocyte cell membranes and provide chondrocyte-matrix interactions vital for cell survival (Goggs et al., 2003), the biochemical assessment of disease progression in cartilage is generally related to proteoglycans and collagen degradation.

The movement of nutrients through cartilage ECM is essential for the functional integrity of synovial joints and the viability of the articulating cartilage (Buckwalter and Mankin, 1998). The ECM itself facilitates the process of diffusion, whereby nutrients and micronutrients, important for cartilage matrix synthesis and maintenance (i.e., glucose, glucose-derived sugars, amino acids, vitamin, and other cofactors), move freely into cartilage matrix. This free diffusion is vital, since chondrocytes are highly susceptible to nutritional deficiency and imbalance, that is, excess quantities of certain nutrients and waste products, such as lactate, particularly during periods of growth, tissue repair, turnover, or remodelling.

Glucose and Glucose-Derived Sugars

Simple sugars, including glucose and sugar-derived biochemicals, provide sources of readily available metabolic energy for chondrocytes and also carbon skeletons for the biosynthesis of proteins, lipids, nucleic acids, and complex polysaccharides. In cartilage, glucose and other hexose sugars serve as building blocks for glycoproteins and glycosaminoglycans that, in addition to their role as structural components of the ECM (Figure 2), fulfil adhesive and informational functions.

Antioxidant Sugar Acids in Cartilage

Hexose sugars also occur in the form of various biologically important derivatives, including sugar acids, sugar alcohols, and deoxysugars, all of which play roles in articular cartilage (Sweeney et al., 1993; Shikhman et al., 2001). Sugar acids are formed by the oxidation of the carbonyl group and/or alcohol group of a simple hexose to a carboxyl group. Oxidation of the terminal aldehyde group yields an aldonic acid, while oxidation of the terminal hydroxyl group forms an uronic acid. Vitamin C or ascorbic acid is a special sugar acid, critical for the growth, development, and enzymatic reactions of cartilage and bone. Vitamin C is a potent antioxidant, and by reducing iron to its ferrous state, it facilitates the hydroxylation of proline and lysine residues to hydroxyproline and hydroxylysine, respectively, within collagen. This activity of vitamin C is essential to the maturation of the collagen molecules (Pacifici and Iozzo, 1988) and the formation of a mechanically robust cartilage matrix. Ascorbic acid, which is transported into chondrocytes by the recently described sodium-dependent vitamin C transporter

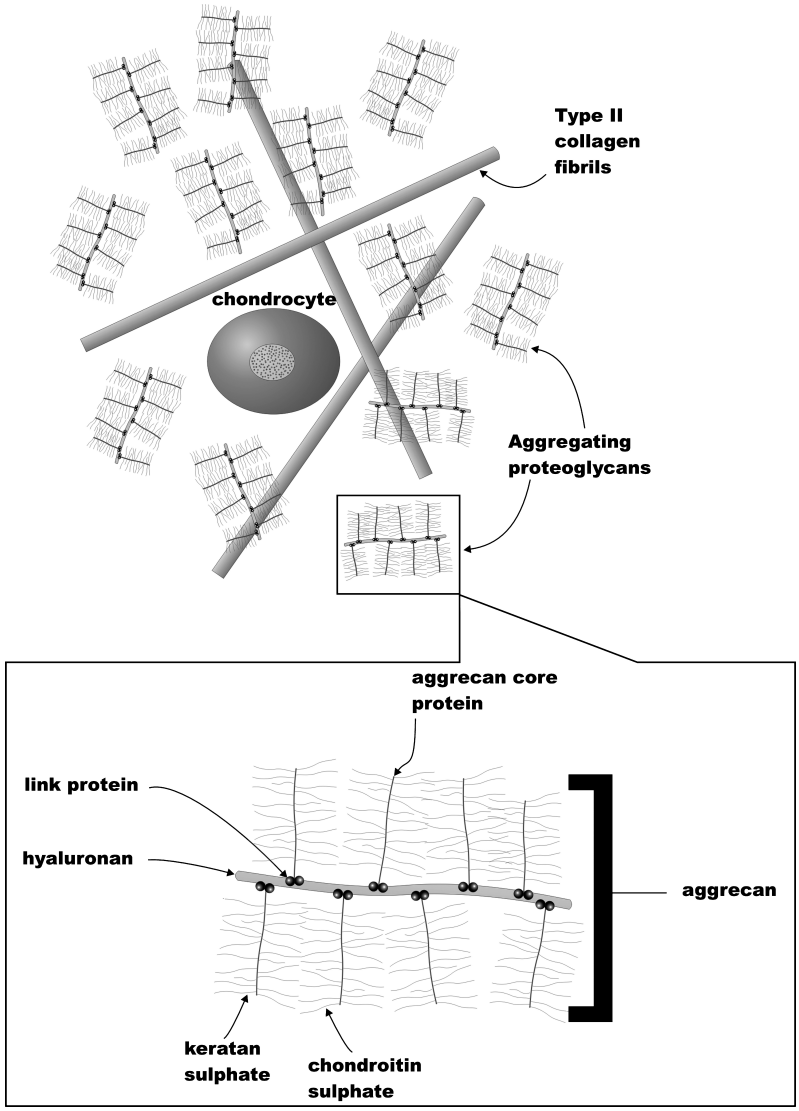

Figure 1 Schematic view of the major components of the extracellullar matrix of articular cartilage, type II collagen and aggregating proteoglycans. The enlarged structure identifies the individual components that make proteoglycans.

(SVCT2), has also been shown to upregulate the expression and activity of prolyl-4-hydroxylase, type II collagen, and aggrecan in guinea pig cartilage (Clark et al., 2002).

There is clinical evidence that antioxidants, such as vitamin C, reduce the risk of cartilage loss and OA progression. A clinical study published in 1996 investigated whether intake of vitamin C might be associated with decreased rates of knee OA (McAlindon et al., 1996). The study recruited 640 participants from the Framingham Osteoarthritis Cohort and found no significant risk association between the incidence of OA and any vitamin or micronutrient. Furthermore, the study concluded that patients with a high vitamin C intake had a reduced risk of developing knee pain and OA progression. The study concluded that high dietary intake of antioxidants, especially vitamin C, may reduce the risk of cartilage loss and OA progression.

Sugar Alcohols in Cartilage

Reduction of the carbonyl group of a simple sugar to an alcoholic hydroxyl yields a sugar alcohol or alditol. The sugar alcohol derived from glucose, sorbitol is believed to be an osmolyte, important for cell volume regulation in many cell types, including articular chondrocytes. Another alditol, inositol is believed

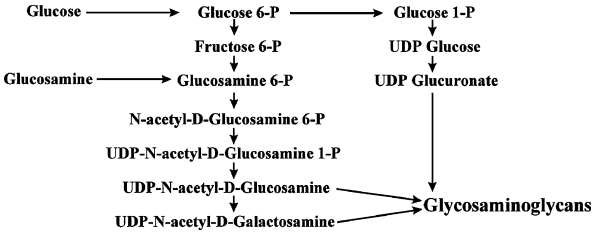

Figure 2 The biochemical pathway that incorporates glucose and glucosamine as primary substrates for the synthesis of matrix glycosaminoglycans (reproduced with permission from Mobasheri et al., 2002b).

to play a similar physiological role in cartilage cells, and in addition, one of its nine stereoisomers, myo-inositol, serves as a component of phospholipids.

Glucose Transporters in Chondrocytes

The glucose transporter (GLUT/SLC2A) family catalyses the entry of hexose sugars into mammalian cells, and thus far, over a dozen GLUT family members have been identified on the basis of sequence similarity (Figure 3; Joost and Thorens, 2001; Joost et al., 2002). These proteins are expressed in a tissue- and cell-specific manner and exhibit distinct kinetic and regulatory properties that reflect their functional and tissue specific roles (Table 1). There is functional evidence from our laboratories and several others for multiple members of the GLUT/SLC2A family in articular chondrocytes (Ohara et al., 2001; Mobasheri et al., 2002; Richardson et al., 2003). These proteins are likely to be involved in the facilitated transport of glucose, fructose, and glucose-derived compounds (e.g., the oxidized form of vitamin C) across the chondrocyte membrane (Figure 4). Some GLUTs may also be involved in intracellular (organellar) glucose transport. There is also some evidence to suggest that GLUTs participate in the transport of glucosamine and N-acetylglucosamine (Rauchman et al., 1992; Cloherty et al., 1995). However, it remains to be seen whether these proteins are also involved in transporting glucosamine and other glucose-derived biochemicals in chondrocytes.

Structure and Symptom Modifying Nutraceuticals

Nutraceuticals may be defined as substances produced in a purified or extracted form that are administered orally to provide or stimulate production of raw materials required for normal bodily functions. A nutraceutical is effectively any substance that is a food, or part of a food, that provides medical or health benefits, including the prevention and treatment of disease. The American Food and Drug Administration (FDA), does not regulate nutraceuticals. Hence, no efficacy data are required or are available for these substances. Nevertheless, laboratory and clinical evidence is emerging that suggests that these compounds may be of benefit in the treatment of OA. Examples of nutraceuticals include the heteropolysaccharides chondroitin sulphate (CS), glucosamine sulphate (GS) and glucosamine hydrochloride (GHCl), essential fatty acids, particularly n-3 polyunsaturated fatty acids (PUFA), and antioxidant sugars (vitamin C).

Chondroitin Sulphate

Chondroitin sulphate is a normal constituent of the major proteoglycan of articular cartilage, aggrecan (Hardingham and Fosang, 1992). In articular cartilage, the hydration of chondroitin sulphate plays an important role in creating a large osmotic pressure that expands the matrix and places the collagen network under tension. CS PGs occupy a solution volume 30–50 times their own dry mass and, as further hydration is prevented by the type II collagen network, a pressure develops within the cartilage that confers its compressive resistance. There is increasing interest in CS as a nutraceutical. Studies in rats and humans have shown that only a small amount of orally administered chondroitin sulphate is absorbed from the gut. In a study conducted by Conte et al. (1991a), the absolute bioavailability of CS has been calculated as 13.2% of the amount ingested, which concurs with the values obtained in a more recent study of 15% and 12% for rats and humans, respectively (Ronca et al., 1998). Subsequently, it appears in plasma and is available for incorporation into proteoglycans in tissues such as cartilage (Verbruggen et al., 1999). The detection of radiolabelled CS in plasma, synovia, and cartilage after oral and intramuscular administration clearly shows that it reaches key tissues following absorption (Conte et al., 1991b; Conte et al., 1995; Uebelhart et al., 1998; Ronca et al., 1998). Demonstrated *in vitro* (Redini et al., 1990) and *in vivo* (Ronca et al., 1998) benefits of exogenous CS include anti-inflammatory effects (reduced macrophage and neutrophil infiltration in soft tissue) and stimulation of hyaluronan synthesis in rabbit synoviocytes (Redini et al., 1990) and human synovial fluid (Ronca et al., 1998). Studies on differentiated human articular chondrocytes have shown that CS significantly increases ^{35}S incorporation rates into aggrecan (Verbruggen et al., 1999). The same investigators used electron microscopy to demonstrate that CS also increases aggrecan aggregate sizes and, in addition, the synthesis of high molecular weight hyaluronan.

There is a considerable number of combination chondroitin and glucosamine products in the human and veterinary marketplaces. The veterinary products are aimed particularly at the equine and canine sectors. There is evidence from *in vivo* studies in rabbits, rats, and dogs (Lippiello et al., 2000; Beren et al., 2001; Johnson et al., 2001), respectively, that these combination products may be more effective than the individual agents are separately; it is suggested that GS and CS may act synergistically.

Glucosamine

Glucosamine is a naturally occurring amino sugar essential for the normal growth and repair of joints and articular cartilage,

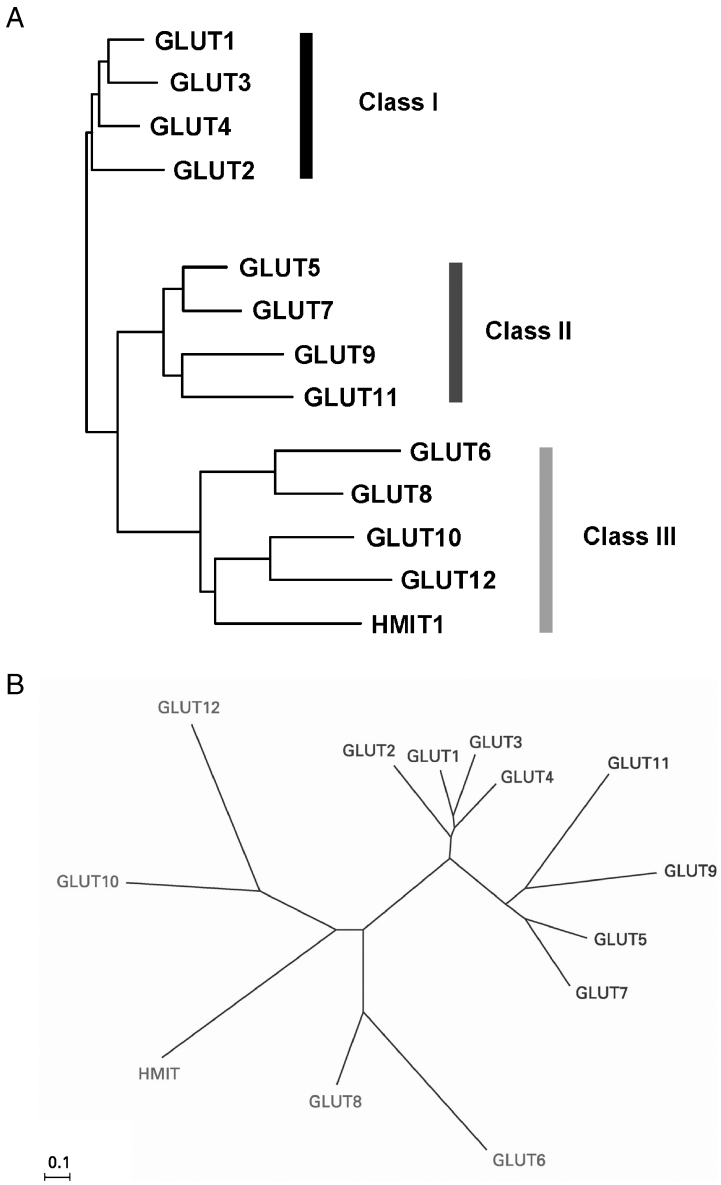

Figure 3 The GLUT/SLC2A family of glucose/polyol transporter facilitators. Panel A shows a dendrogram of a multiple alignment of all members of the extended GLUT family (reproduced from Joost and Thorens (2001) with kind permission of the authors and Taylor and Francis Ltd.). Panel B shows an unrooted radial phylogram drawn from a multiple sequence alignment of the 13 members of the human GLUT/SLC2A family. The three classes of GLUT proteins are color-coded: **blue, class I**; **red, class II**; **green, class III**. The same color-code is also used in Table 1. The scale bar represents 0.1 substitutions per amino acid position. HMIT, H^+-coupled myo-inositol transporter. Reproduced from Wood and Trayhurn (2003) with permission of the authors.

where it is a normal constituent of GAGs, both in the ECM and in the synovial fluid. Glucosamine compounds, particularly the sulphated form and initially extracted from animal products, are now widely available in a pure crystalline form and have been used as nutraceuticals for human OA in Europe for more than a decade. Since then, many studies have been conducted on the *in vitro* and *in vivo* effects of GS, but its clinical efficacy and mechanism of action is still a matter of debate. It is clear that there is substantial interest in GS from both the research and industrial communities.

Numerous clinical trials of varying lengths in humans (Pujalte et al., 1980; Reichelt et al., 1994; Noack et al., 1994; Reginster

Table 1 Isoforms of the GLUT/SLC2A family of facilitative sugar/polyol transporters

Isoform	Previous name	Class	Tissue localization	Present in chondrocytes	Insulin sensitive	Functional characteristics (transport)
GLUT1	—	I	Ubiquitous: erythrocytes, brain, cartilage	Yes	No	Glucose
GLUT2	—	I	Liver, pancreas, intestine, kidney	No	No	Glucose (low affinity); fructose
GLUT3	—	I	Brain, cartilage	Yes	No	glucose (high affinity)
GLUT4	—	I	Heart, muscle, adipose tissue, brain	No	Yes	Glucose (high affinity)
GLUT5		II	Intestine, testis, kidney	Yes (mRNA)	No	Fructose; glucose (very low affinity)
GLUT6	GLUT 9	III	Brain, spleen, leukocytes	Yes	No	Glucose
GLUT7		II	n.d.	n.d.	n.d.	n.d.
GLUT8	GLUT X1	III	Testes, brain and other tissues	Yes (mRNA)	No; (yes in the blastocyst)	glucose
GLUT9	GLUT X	II	Liver, kidney, cartilage	Yes	n.d.	n.d.
GLUT10		III	Liver, pancreas	Yes (mRNA)	No	Glucose
GLUT11	GLUT 10	II	Heart, muscle	Yes (mRNA)	No	Glucose (low affinity); fructose (long form)
GLUT12	GLUT 8	III	Heart, prostate, muscle, small intestine, adipose tissue, cartilage	Yes	Yes	n.d.
HMIT		III	Brain	n.d.	n.d.	H^+/myo-inositol

GLUT/SLC2A family members facilitate the transport of glucose, galactose, fructose, and glucosamine into mammalian cells. Adapted from Mobasheri et al., 2002b and Wood and Trayhurn, 2003.

et al., 2001; Pavelka et al., 2002) and animals, including purebred dogs, horses, and mixed-breed dogs (McNamara et al., 1996; Hanson et al., 1997; Canapp et al., 1999), have sought to determine the efficacy of GS as a treatment option for OA. Many of the results presented are conflicting and inconclusive. Some of these studies have shown significant symptom modifying effects, improvements in arthritis indices, and histopathological lesion severity, coupled with an attractive safety profile, while others have shown little or no response (Noack et al., 1994; Houpt et al., 1999; Rindone et al., 2000). Glucosamine has been favorably compared with conventional therapies, particularly non-steroidal anti-inflammatory drugs (NSAIDs) (Muller-Fassbender et al., 1994; Qiu et al., 1998). A meta-analysis and quality assessment of 15 double-blind, randomized, placebo-controlled clinical trials of glucosamine and chondroitin nutraceutical compounds has evaluated the efficacy of these agents to treat OA (McAlindon et al., 2000). All but one of these trials were classified as positive, and the studies collectively demonstrated moderate effects for glucosamine and large effects for chondroitin (Figure 5). However, quality scoring (on a scale of 0 to 100; 100 being the highest quality) showed major deficiencies in descriptions of randomization, blinding, and completion rates. A thorough quality assessment detected significant publication bias, poor trial design, including small sample size (Pujalte et al., 1980; Vaz 1982), lack of long-term follow up (Reichelt et al., 1994), and potential bias, resulting from commercial sponsorship of research (Lippiello, 2003) among many of the published studies. These methodologic problems are, at best likely to lead to exaggerated estimates of benefit. In addition, the trials in the meta-analysis only measured symptoms. Inferences, therefore, cannot be drawn about the potential of these compounds to affect the pathologic progression of OA (Hochberg et al., 2000). The authors of these various studies have concluded that only a moderate degree of nutraceutical efficacy could be reliably shown to exist.

Many of the studies that demonstrate favorable results for glucosamine use scoring indices, such as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), in order to quantify what are essentially subjective assessment criteria for patient improvement. These indices seek to reduce the subjective nature of patient assessment of pain and joint function and have been validated for that purpose. However, these indices can never be truly objective, apply to small periods of time (typically the last 48 hrs), and cannot be applied to anything other than humans. Thus, in order to study animals, other methods have been used, including haematological and biochemical analysis (McNamara et al., 1996), radiography (Canapp et al., 1999), and scintigraphy/fluoroscopy (Hanson et al., 1997). There are a number of studies that use noninvasive, quantitative, and objective methods, including kinetic/kinematic gait and force plate analysis, for assessing limb and joint function (Bertram et al., 2000), and drug efficacy (Daumen-Legre et al., 1993; Budsberg et al., 1999). These techniques, although principally developed for use in humans (Sakauchi et al., 2001; Al-Zahrani et al., 2002), are also being used to study joint disease in animals both in experimental and clinical settings (Botrel et al., 1994; Moreau et al., 2003).

One such recent double-blind study that involved 71 osteoarthritc dogs compared the efficacy, tolerance, and ease of administration of two routinely prescribed NSAIDs: carprofen and meloxicam and a combination GS-CS nutraceutical. The authors found significantly improved ground reaction forces of the arthritic joints following NSAID treatment, but not following nutraceutical therapy (Moreau et al., 2003). This was

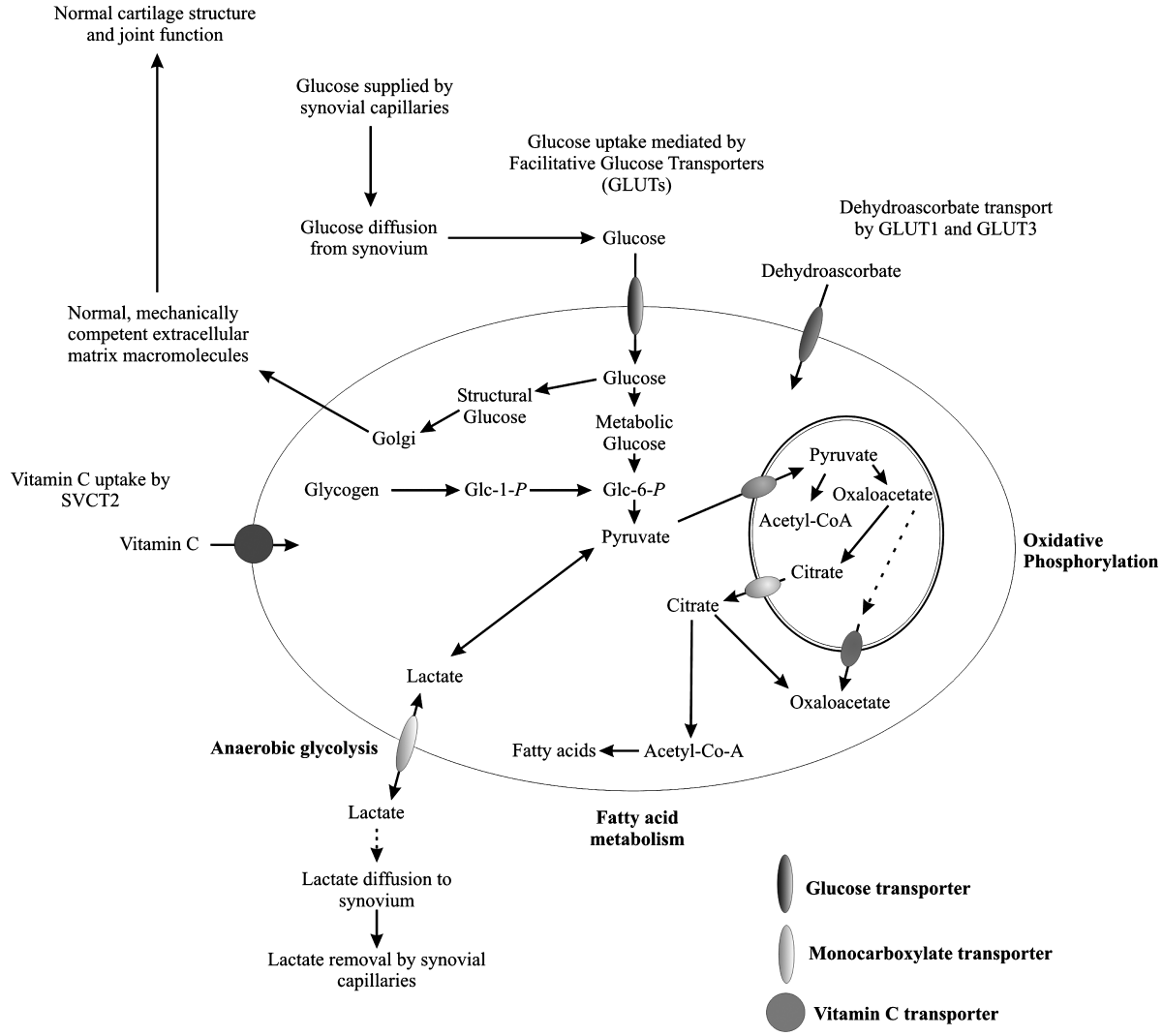

Figure 4 Glucose and vitamin C transport in articular chondrocytes. Glucose is delivered to the chondrocyte via the synovial microcirculation and taken up by GLUT proteins. Intracellular glucose is accumulated in 2 distinct pools: the metabolic pool used for glycolysis and the structural pool used for synthesis of ECM macromolecules. Lactate efflux from chondrocytes may be facilitated by monocarboxylate transporters (i.e., MCT-1; Mobasheri et al., 2002b) and vitamin C may be transported by SVCT2 (Clark et al., 2002) and possibly also by GLUT1 or GLUT3 (Agus et al., 1997; Mobasheri et al., 2002b).

compared with subjective assessments both by owners and veterinary orthopaedic surgeons. It was concluded that both NSAIDs improved the articulations of the arthritic joints, while the nutraceutical did not.

Two significant issues for any new therapeutic compound to contend with are those of bioavailability and pharmacokinetics. A series of experiments in rats, dogs, and humans, conducted since the mid 1980s have shown that up to 90% of orally administered GS is absorbed from the gastrointestinal tract (Setnikar et al., 1984; Setnikar et al., 1986; Setnikar et al., 1993). This does depend somewhat on the species studied, since this figure relates only to humans. As expected, the maximum plasma concentrations are achieved following parenteral administration, while plasma levels following oral administration are somewhat lower, typically only a quarter of that actually absorbed, most likely due to first pass effect of the liver (Setnikar et al., 1993). GS is protein bound in plasma; in this form, it has a long half-life of 2.9 days. Many tissues, including articular cartilage, show active uptake of GS from plasma (Setnikar et al., 1986). Thus, it is clear that GS does reach the intended site of action and potentially, in sufficient quantities, if it is to be of value.

Experiments in a plethora of cell types from a variety of species have attempted to elucidate the mechanism of action

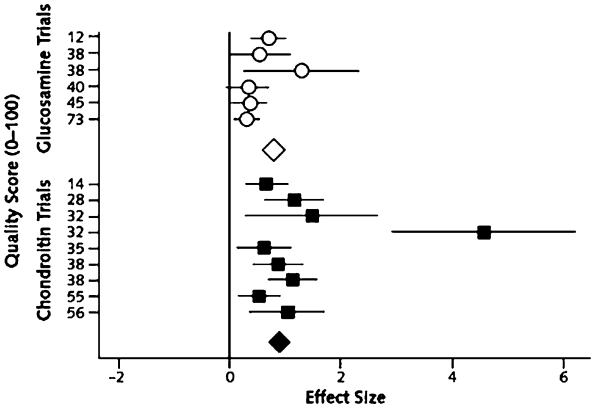

Figure 5 Meta-analysis of placebo-controlled trials of glucosamine and chondroitin in OA. The white circles represent the effect size of each glucosamine trial; 0 represents no effect relative to placebo. Horizontal lines represent 95% confidence intervals (CIs). The white diamond represents aggregate results (with 95% CI) for glucosamine trials. The black circles and diamonds represent the same features for chondroitin. Reproduced from Hochberg et al., 2000.

of GS (Oegema et al., 2002; Orth et al., 2002). There is now a body of evidence that suggests that GS possesses an unusual combination of anti-inflammatory, cell stimulatory, and disease process modifying actions. The anti-inflammatory properties are due to dose-dependent inhibition of inducible nitric oxide synthase (iNOS), induction by both lipopolysaccharide (LPS) and interleukin 1-beta (IL-1β (Meininger et al., 2000; Shikhman et al., 2001), prevention of interleukin-6 (IL-6) generation, and cyclo-oxygenase-2 (COX-2) inhibition, as well as prostaglandin E_2 (PGE_2) production (Gouze et al., 2001).

There is much uncertainty as to the intracellular signalling pathway employed by GS for these properties. One study demonstrated inhibition of nuclear factor kappa B (NF-κB) (Gouze et al., 2002), while another did not, despite using similar experimental protocols (Shikhman et al., 2001) in rat and human chondrocytes, respectively. These latter authors also found that the IL-1β effect; inhibition effect by GS was not associated with the activation of any of a range of other common signalling pathways, including mitogen-activated protein kinase (MAPK), cJun-N-terminal kinase (cJNK), extracellular signal-regulated kinase (Erk), or p38 kinases. A further study also revealed that, in human chondrocytes, glucosamine sulphate inhibits NF-κB activation, although in this case the cells were obtained from osteoarthritic tissue (Largo et al., 2003). A putative glycosylphosphatidylinositol (GPI) linked protein has been suggested to be part of the GS signalling/regulation pathway (Sandy et al., 1998). The pathway may not be clear, but GS undoubtedly operates at transcription level (Jimenez et al., 1997; Wang et al., 1998), which may explain the slow adaptive response to GS therapy suggested by Giordano et al. (1996).

Another anti-inflammatory action mediated by GS is the increased expression of IL-1RII receptors. These receptors bind the inflammatory cytokine, IL-1β but do not relay the message intracellularly, thereby modulating the concentration of active cytokine. Furthermore, GS is able to inhibit expression and activation of key matrix-degrading enzymes, including aggrecanase and matrix metalloproteinase (MMP-3—stromelysin 1) (Sandy et al., 1998; Gouze et al., 2001 respectively). Additionally, GS increases the production of key ECM components, including aggrecan (Jimenez and Dodge, 1997; Dodge and Jimenez, 2003). The modulation of enzymes by GS at an mRNA level also potentially explains the reversal of proteoglycan synthesis following GS administration to IL-1β treated chondrocytes. IL-1β inhibited galactose-b(beta)-1,3-glucuronosyltransferase (GlcAT-1) expression and activity, an enzyme key to the synthesis of GAG chains (Gouze et al., 2001), in a dose-dependent reversal by GS, which is an effect also noted by Bassleer and colleagues (1998). GS is the preferred substrate for GAG production (Noyszewski et al., 2001) and is selectively incorporated following systemic administration (Setnikar et al., 1984). GS also demonstrates anti-inflammatory properties in acute synovitis models (Canapp et al., 1999) and has anabolic effects on both synoviocytes and chondrocytes, for instance, by enhancing protein synthesis (Jimenez and Dodge, 1997; Piperno et al., 2000).

Although GS therapy has a good safety profile, a possible link with insulin resistance should be addressed. One potential problem with a glucose-derived nutraceutical, such as glucosamine, is the potential disturbance of systemic glucose metabolism. A number of reports suggest that GS induces insulin resistance in fibroblasts (Marshall et al., 1991) in the absence of high glucose or glutamine and in other cell types, including myocytes (Robinson et al., 1993; Patti et al., 1999) and adipocytes (Chen et al., 1997; Hawkins et al., 1997). Additionally, findings of one study suggest that intravenous GS could induce insulin resistance in normoglycaemic but not hyperglycaemic rats (Rossetti et al., 1995). Further studies involving glucosamine infusions have shown that this compound causes hyperglycaemia, raised the threshold level for glucose-induced insulin secretion (Monaumi et al., 2000), and can induce insulin resistance *in vivo* via activation of the hexosamine pathway (Virkamaki et al., 1997). This pathway is a glucose disposal route, which acts by removal of fructose-6-phosphate via combination with glutamine and subsequent convertion to hexosamine. Recently, however, when two strains of rats, both highly susceptible to induced insulin resistance were challenged by GS, no insulin resistance induction with GS, CS, or combinations of the two could be demonstrated (Echard et al., 2001).

It is difficult to draw reliable conclusions from these contrasting studies, as the conflicting data may represent differences between species, cell type, or model. Nevertheless, there appears to be a significant body of evidence that glucosamine can induce insulin resistance under certain conditions and, as such, should be considered when making judgements about the safety of this compound for particular at-risk patients.

Further conflicting data have been obtained from studies investigating the effect of glucosamine on chondrocyte metabolism *in vitro*, in particular, effects on lactate production, protein

synthesis, and proteoglycan production (Ilic et al., 2003; Anderson et al., 1999; De Mattei et al., 2002). Overlapping ranges of glucosamine concentrations (from 0.2 mM to 55 mM) were used in all three studies, but while one group concluded that at concentrations of up to 10 mM no detrimental effects could be demonstrated (Ilic et al., 2003), two other groups have suggested that, at concentrations down to 100 μg/ml (approximately 0.2 mM), GS produced significantly detrimental effects on chondrocyte metabolism (Anderson et al., 1999; De Mattei et al., 2002). Again, there were inconsistencies between the three studies: two involved bovine articular chondrocytes explants (De Mattei et al., 2002; Ilic et al., 2003), while the third used isolated canine articular chondrocytes (Anderson et al., 1999). Also, De Mattei et al. (2002) used GHCl, while the others used pure glucosamine. Without direct repetition of the experimental protocols, it is not possible to draw strong conclusions from these publications. However, it does clearly indicate the difficulty facing clinicians attempting to make an informed rational decision regarding the safety of these compounds.

The other principal concern regarding glucosamine, that of immunosuppression, is potentially a double-edged sword, since at a local level this might be of benefit in inflammatory conditions, while systemic immunosuppression may compromise the general well-being of the patient. Glucosamine has been found to dose-dependently suppress the activation of T-lymphoblasts and dendritic cells *in vitro* (Ma et al., 2002) and to dose-dependently suppress neutrophil superoxide generation, lysozyme release, and complement induced chemotaxis (Hua et al., 2002). Both sets of authors suggest that these effects might be of benefit in the treatment of inflammatory disease, but do concede that, until carefully controlled studies are undertaken, the risk to patient immune status cannot be assessed.

Amino Acids

Amino acids are essential for the synthesis of proteins and ECM, but very little is known about their transport into chondrocytes. Many different families of amino acid transporters have been identified in epithelia and other cell types (Verrey et al., 1999, 2000, Mann et al., 2003), but thus far, there is only one published report on the transport and uptake of amino acids into bovine chondrocytes (Barker et al., 1999). These authors examined proline, glycine, glutamine, leucine, and tryptophan uptake and convincingly demonstrated the presence of multiple transport systems, including those systems known as A, Gly, and L; system A is a Na^+ dependent proline transporter, system Gly is Na^+ and Cl^-; dependents and the ubiquitously expressed system L is responsible for leucine and tryptophan transport. The authors concluded that transport of leucine and tryptophan into bovine chondrocytes occurs solely by system L, but with a higher affinity than the conventional L system. It should be emphasized at this point that the amino acid transport systems previously mentioned have been renamed since the introduction of a new nomenclature (Mann et al., 2003).

It is clearly impossible to draw strong conclusions on the role of amino acids in cartilage biology on the basis of this single study. However, it seems likely that cartilage has specific transport systems for amino acids in the same way it has for glucose, implying that amino acid uptake and metabolism is of particular significance for chondrocyte metabolism and for the maintenance of cartilage integrity, as might be expected. Further studies are required to determine whether the expression of amino acid transport systems is altered in OA and in a state of malnutrition, since a better understanding of pathological alterations of amino acid metabolism may provide potential therapeutic avenues.

Essential Fatty Acids

The essential fatty acids are a particular group of polyunsaturated fatty acids (PUFAs), fatty acids that contain more than one carbon-carbon double bond within their structure. The two principle essential fatty acids are linoleic acid (18:2 n-6 LA) and α-linolenic acid (18:3 n-3 LnA), the n- designation referring to the position of the double bond relative to the omega carbon atom (the methyl carbon at the distal end of the chain). Arachidonic acid (eicosatetraenoic acid), an n-6 fatty acid, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), n-3 fatty acids, may be derived from dietary LA and LnA, respectively, via desaturation and elongation. The essential fatty acids are components of cell membranes, involved in lipid transport, and serve as precursors to the eicosanoid hormone family, which regulates inflammatory processes.

The n-3 and n-6 fatty acids compete for incorporation into phospholipids and as substrates for COX -1 and -2 and 5- lipoxygenase (LOX). While the metabolism of both types of fatty acids leads to eicosanoid production, a higher proportion of n-6 fatty acid within cell membranes is believed to promote the production of the inflammatory prostaglandins, leukotrienes, and thromboxanes. Arachidonic acid, in particular, is a precursor of pro-inflammatory eicosanoids. While many studies have reported the potential anti-inflammatory and therapeutic effects of n-3 fatty acids in RA (for review see Rennie et al., 2003), a disease that is associated with extensive inflammation of the synovium and cartilage, there have been relatively few studies that indicate the role of an n-3 to n-6 imbalance in pathogenesis of OA.

An association between lipid composition and tissue pathology in OA articular cartilage was demonstrated in a study by Lippiello et al. (1991). Specifically, the study indicated that the severity of OA cartilage lesions is linked to a higher proportion of the n-6 fatty acid, arachidonic acid. Two of the local eicosanoid hormones produced from n-6 fatty acids, PGE_2 and leukotriene B4, are considered key mediators of inflammation in arthritis, and consistent with the previous study, it has been shown that the PGE_2 levels produced spontaneously by OA cartilage are highly elevated (Amin et al., 1997). The levels were found to be elevated, even relative to healthy cartilage stimulated directly by the pro-inflammatory cytokines that promote

cartilage degradation This latter study also revealed that COX-2, which is not expressed constitutively, is up-regulated in chondrocytes from cartilage affected by OA as well as that cartilage affected by RA, lending further support to the hypothesis that OA has a strong inflammatory component.

More recent studies (Curtis et al., 2000, 2002) have provided direct evidence that n-3 fatty acid supplementation can reduce or abrogate the inflammatory and matrix degradative response elicited by chondrocytes during OA progression. As n-3 and n-6 fatty acids become incorporated into cell membranes, their ratio within the membrane determines whether or not the cell is primed for a pro-inflammatory response (high n-6). By preincubation of chondrocytes with different fatty acids, Curtis et al. (2000) showed that this membrane ratio could be modified to render the cell less pro-inflammatory, concomitant with down-regulation of COX-2, chondrocyte derived pro-inflammatory cytokine mRNA, and cytokine induced matrix degradation. This study was extended (Curtis et al., 2002) to show that OA cartilage proteoglycan and collagen degradation and inflammation could also be reduced by exposure to n-3 fatty acids. The abrogation of expression of key mediators of cartilage degradation in OA, such as MMP-3, MMP-13, COX-2, 5-LOX, tumour necrosis factor alpha (TNF-α), and IL-1, suggest that n-3 fatty acid supplementation, resulting from dietary intake of fish oil, may be an extremely powerful approach to alleviating symptoms and progression of OA. Thus, taking a systemic approach, the imbalance of fatty acid intake may place an individual in a pro-inflammatory state (Lands et al., 1992). Not only are n-6 PUFA, like arachidonic, acid preferentially metabolized by COX, but additionally, n-6 PUFA derived eicosanoids are more efficacious receptor agonists (Lands, 1992). These combined effects render individuals on Western diets more susceptible to inflammatory conditions, since Western diets are abundant in n-6 PUFA yet low in n-3 PUFA. This state of affairs has resulted from changes in consumption of particular plant oils during the last century. The western dietary ratio of n-6:n-3 has been estimated to range between 25:1 (Simopoulos, 1991) and 10.6:1 (Kris-Etherton et al., 2000). It is clear that the ratio is far from optimal for reduction of susceptibility to inflammatory disorders (Simopoulos et al., 1999).

Given the concern regarding the over-supply of n-6 PUFA, typical of western diets, it is no surprise that the volume of research into the potential benefits of n-3 PUFA dietary supplementation is considerable. However, as stated earlier, most of the studies relate to RA, a disease that is not localized to the articular joint alone. Several studies have shown consistently beneficial effects of n-3 supplements on joint disease (Belch et al., 1988; Cleland et al., 1988; Kremer, 1991; Nielsen et al., 1992), the majority of which are clinical studies, and such treatment is continually under review (Volker and Garg, 1996). Furthermore, combining high n-3 fatty acid intake with low arachidonic acid intake (high fish oil, low meat consumption) is postulated (Adam et al., 2003) to provide an augmentation of the beneficial effects observed using n-3 fatty acid supplementation alone in RA.

In summary, n-3 PUFA reduces the load of pro-inflammatory eicosanoids and modulates key inflammatory factor production at a gene transcription level. Numerous reports exist concerning n-3 PUFA supplementation reducing or abolishing production of inflammatory mediators and enzymes, including interleukin-1 alpha (IL-1α) and IL-1β, TNF-α, COX, and LOX (both *in vitro* and *in vivo* studies; Lee et al., 1985; Endres et al., 1989; Caughey et al., 1996; Mantzioris et al., 2000; Curtis et al., 2000; Curtis et al., 2002). These experimental studies show that n-3 PUFA supplementation can specifically affect molecular mechanisms regulating the inflammatory mediators and catabolic factors involved in pathological cartilage degradation. In addition, a mega-analysis study of seven published papers and three clinical trials of the effects of dietary fish oil in RA has revealed that fish oil supplementation significantly reduces tender joint count and morning joint stiffness compared with heterogeneous control oils (Fortin et al., 1995). In summary, there is good evidence that n-3 dietary supplementation is of benefit to human patients suffering from joint disease, although no comparable studies exist in domestic animals. This is another potentially important field of clinical research that remains to be undertaken.

Green Lipped Mussels

There is considerable interest in the potential anti-inflammatory properties of extracts from *Perna canaliculus* (the New Zealand Green Lipped Mussel (GLM)). The first reports on this mollusk appeared in the mid-1970s, and a number of theories have been purported to explain the effects observed, some supported by more experimental evidence than others. It is clear, however, that industry is now backing research and pursuing the commercial potential of this species (Bierer and Bui, 2002). Early researchers reported that freeze-dried extracts of GLM inhibited experimentally induced inflammation and were also gastroprotective (Rainsford and Whitehouse, 1980). This is significant in two ways: not only were GLM extracts anti-inflammatory in their own right, but it has been proposed that GLM might be used as an adjunct to NSAID therapy, as gastric ulceration is a typical side effect of long-term NSAID therapy. The efficacy of this proposal needs to be confirmed in a clinical study of the combined therapy. The reported findings of anti-inflammatory and gastroprotective properties have since been duplicated (Bui and Bierer, 2001), but there is little consensus as to the method of action of the compounds within the GLM extract or, indeed, exactly which compounds are responsible for the beneficial effects observed. Different studies have identified the importance of either lipids or high molecular weight polysaccharides in their effect (Couch et al., 1982; Miller et al., 1993), although recent research, again, points to a lipid rich extract, particularly the PUFA component (Whitehouse et al., 1997). GLM has abundant n-3 PUFA, which makes up greater than 30% (Murphy et al., 2002) of the total fatty acids. N-3 PUFAs have been consistently linked with alleviating the symptoms of arthritis and other inflammatory disorders (Whitehouse et al., 1997; James

and Cleland, 1997; James et al., 2000). They are thought to act as inhibitors of both cyclo-oxygenase and lipoxygenase pathways of arachidonic acid oxygenation.

Green Tea Polyphenols

Green tea, produced from the dried leaves of the plant *Camilla sinesis*, has been consumed both as a beverage and for medicinal purposes for 5000 years in China and Japan (Kaegi, 1998). Recently, considerable attention has been focused on a group of polyphenolic compounds called catechins, a number of which are to be found in green tea. The most abundant of these is (-)-epigallocatechin gallate (EGCG), but others include (-)- epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epicatechin (EC). These compounds are well known as antioxidants (Cotelle et al., 1992; Dreosti, 2000), but there is evidence that these polyphenols also have antimicrobial (Ikigai et al., 1993; Rasheed and Haider, 1998), immunostimulatory (Hu et al., 1992), and anticarcinogenic properties (Katiyar and Mukhtar, 1996; Ahmad et al., 1997, 2000).

A number of studies have demonstrated the anti-inflammatory properties of the catechins. In addition, they have a number of other properties that make them excellent candidates for the nutraceutical prophylaxis and treatment of OA. Several groups have revealed that green tea polyphenols inhibit the induction of iNOS by IL-1β and ultimately the production of nitric oxide (NO). This free radical is a key mediator of a number of the pathophysiological processes that occur in OA (Goggs et al., 2003). This inhibition results from the suppression and blockade of the downstream transcription factor, NF-κB, through the modulation of the NF-κB inhibitor, IκBa (Lin and Lin, 1997; Yang et al., 2001; Singh et al., 2002).

This may be of indirect prophylactic benefit by reducing the levels of NO, thus diminishing its detrimental effects on cartilage integrity. A second indirect effect of potential therapeutic value is that observed by Demeule et al. (2000) and Annasi et al. (2002). Both studies revealed that catechins from green tea are potent inhibitors of MMPs. These enzymes are responsible for the breakdown of cartilage matrix components and for the destabilization of the cell-fibre-matrix network (Cao et al., 1999). Unfortunately, since both studies were conducted using glioblastoma cells and, to date, there are no publications relating to chondrocytes, this potential link must be treated with caution. Clearly, further research is required in this area.

Using the collagen-induced murine polyarthritis model of OA, which produces a disease similar to RA, catechins were found to significantly reduce incidence of arthritis measured by clinical assessment and histological examination. Additionally, a marked reduction in the expression of inflammatory mediators, including TNFα, COX-2, and interferon gamma (IFN-γ), was observed in the polyphenol supplemented mice (Haqqi et al., 1999).

Polyphenols also may well have other chondroprotective properties. Adcocks et al. (2002) showed that catechins from green tea inhibited the enzymatic degradation of collagen II and proteoglycan in both bovine and human cartilage. Four of the polyphenolic compounds were tested and found to have overlapping effects, i.e., no one polyphenol individually produced all the effects observed. This is significant from the viewpoint of nutraceutical preparations, which would need to contain several catechins. Importantly, no toxic or non-specific effects judged on the basis of lactate production and rates of proteoglycan synthesis and incorporation were detected.

Perhaps the most notable problem facing the development of the green tea polyphenols as nutraceuticals is that of bioavailability. Plasma concentrations rarely exceed 1 μM after consumption of 10–100 mg of a compound (Scalbert and Williamson, 2000), and with a plasma half-life of just 1 to 2 h, repeated administration would be required to maintain such a plasma level. Little is currently known about the availability or activity of polyphenol metabolites, but such data would be essential for any commercial development of these green tea constituents.

Micronutrients in Cartilage

It has been suggested that diets deficient in trace elements, such as zinc, magnesium, selenium, and copper, may lead to arthropathy. Although little is known about heavy metal transport in chondrocytes, we have briefly summarized the evidence for the role of these micronutrients in connective tissue function, since deficiencies in these trace elements has been linked to the development of OCD in domestic and food producing animals. It is also important to note that excess quantities of these micronutrients result in toxicity (particularly copper).

Magnesium

Body magnesium is primarily located in bone and cartilage (Schaafsma et al., 2001), and up to a third of the magnesium in the body is intracellular (Rude and Olerich, 1996). Complexed with adenosine tri-phosphate (ATP), magnesium participates in enzyme reactions, such as cyclic adenosine mono-phosphate (cAMP) formation, phosphorylation events, protein synthesis, and nucleic acid synthesis. Magnesium is important for many physiological functions; the activity of numerous enzymes depends on the provision of dietary magnesium, and the deficiency of this divalent cation generally enhances chondrocyte vulnerability to toxic insult. Magnesium and calcium supplementation is thought to be associated with higher bone mineral density and higher total bone mass (Yano et al., 1985). Magnesium is also important for cartilage and tendon function (Stahlmann et al., 1995; Shakibaei et al., 1996). It has been demonstrated that magnesium deficiency leads to joint weakness and limping in immature beagle dogs (Stahlmann et al., 2000) and quinolone cytotoxicity in developing rat cartilage (Forster et al., 1996; Forster et al., 1997; Vormann et al., 1997). Quinolone antibiotics commonly formed stable chelate complexes with di- or tri-valent cations and may be essential for their antimicrobial binding of DNA gyrase. Quinolone-induced arthropathy is most likely due to a deficit of available magnesium due to the chelation of the metal ions

by the quinolone. Magnesium deficiency in articular cartilage may impair chondrocyte-matrix interactions, since magnesium is required for the function of integrins, cell surface receptors that mediate cell attachment to ECM components and act as molecular conduits for inside-out and outside-in signal transduction and communication in focal adhesion sites (Ruoslahti and Pierschbacher, 1987). In chondrocytes, integrins are important for cartilage development and chondrogenesis (Shakibaei, 1998).The inhibition of integrins by means of function inhibiting antibodies is able to interfere with chondrogenesis (Zanetti et al., 1990; Shakibaei, 1998) and induces apoptosis in chondrocytes (Shakibaei et al., 2001). Since integrins depend on extracellular divalent cations, such as magnesium, for their function, it is suggested that dietary magnesium deficiency impairs the expression and activity of these receptors causing cell damage, cell death, and ECM degeneration, which may ultimately lead to cartilage damage and joint lesions (Stahlmann et al., 1995). Little is known about magnesium transport in chondrocytes, and the plasma membrane system(s) responsible for magnesium uptake in these cells have yet to be identified.

Zinc

Zinc has long been known to play important roles in ossification and mineralization; it is an important component of matrix metalloproteases and is required for the binding of collagen and procollagens by matrix proteins (Rosenberg et al., 1998). Dietary zinc deficiency has been shown to interfere with skeletal metabolism and development in chicks (Westmoreland and Hoekstra, 1969a,b), thoroughbred horses (Bridges and Harris, 1988), and growing rats (Rossi et al., 2001). Zinc deficiency has also been shown to depress growth and cartilage metabolism in a separate study by Bolze and colleagues (1987). Conversely, excess dietary zinc intake can result in lameness and cartilage fractures in foals, leading to a syndrome similar to OCD (Bridges and Moffitt, 1990).

The mechanisms of zinc action are not well understood, but the joint changes induced by zinc deficiency may be the result of reduced growth plate activity mediated by impairment of the insulin-like growth factor 1 (IGF-I) system (Rossi et al., 2001). Other studies have suggested that the stimulatory effects of zinc on cartilage growth are due to effects on proliferating chondrocytes and by delaying the onset of mineralization, possibly by modulating the charge density of the proteoglycans (Kirsch et al., 2000; Rodriguez and Rosselot 2001). The addition of zinc compounds to chondrocyte cultures increased the hydrodynamic size of proteoglycans and the production of collagen (Koyano et al., 1996; Rodriguez and Rosselot, 2001). Zinc transport mechanisms in chondrocytes are yet to be identified.

Copper

Copper is of particular interest with regard to OCD, considered to be the most important equine developmental orthopaedic disease (McIlwraith, 1986). It is agreed that this disease is multifactorial. Various nutritional factors have been implicated, including copper, which has been considered a potential cause for twenty years, when Bridges et al. (1984) observed osteochondrosis (OC)-like lesions in copper deficient foals.

Copper is an essential mineral element, which serves as a cofactor in a number of important enzymes both in cartilage and other tissues, including superoxide dismutase, cytochrome oxidase, and particularly relevantly lysyl oxidase. This enzyme is required for the formation of pyridoline cross-links between collagen fibrils. It was suggested that copper deficiency via inadequate lysyl oxidase leads to poorly cross-linked, weakened cartilage, susceptible to subsequent fragmentation. Much is known about dietary copper interactions and the principle plasma transport protein ceruloplasmin, but little work has focussed on cellular transport mechanisms. A potential chondrocyte copper transport protein has been identified, however. The high molecular weight cartilage matrix glycoprotein (CMGP) shares some sequence homology and enzymic activities with ceruloplasmin. CMGP is localized in mitochondrial, cytosolic, and plasma membrane fractions and is the principle chondrocyte protein labelled by ^{67}Cu in *in vitro* uptake studies, suggesting that this protein is directly involved in chondrocyte copper transportation (Fife et al., 1994). Dietary cation and anion imbalances resulting in acidotic diets have been shown to induce dyschondroplasia (Cook et al., 1994). The dyschondroplasia observed is primarily in the tibia and is the result of decreased growth plate cartilage degradation.

The role of copper in equine OCD has been further elucidated through experimental induction (Bridges and Harris 1988), epidemiological studies (Knight et al., 1985; Gabel et al., 1987), and dose-response studies involving foals fed high copper diets (Knight et al., 1990). These studies lead to higher recommendations for copper in horse feed, which increased from 10 mg/kg in 1989 to 25 or even 50 mg/kg (Hurtig et al., 1993; Lewis 1995, respectively).

Copper is reported to have anti-arthritic effects (Rosenstein and Caldwell 1999; Caldwell, 1999), and there are some reports that suggest potential mechanisms for these, although it should be noted that there is some disagreement between the published data. Studies in 1996 (Pasqualicchio et al., 1996; Davies et al., 1996) demonstrated the stimulatory effects of copper supplementation to chondrocytes *in vitro* on proteoglycan synthesis and abrogation of proteoglycan depletion following exposure to inflammatory synovium. However, a more recent study (Heraud et al., 2002) failed to reproduce this result, but did demonstrate a concentration dependent increase in collagen synthesis with a coincident upregulation of collagen II compared to collagen I. Importantly, although both studies used similar methods for monitoring proteoglycan and collagen synthesis, other aspects of experimental protocol differed significantly, potentially explaining the lack of comparable results.

However, following recent studies involving percutaneous liver biopsy of pregnant mares (Pearce et al., 1998a), the role of copper in the pathogenesis of OCD has been de-emphasised

(Jeffcott and Davies, 2000), and relatively low pasture levels of 4.4–8.6mg/kg deemed sufficient for healthy cartilage development (Pearce et al., 1998b). It appears that equine hepatic copper storage is relatively unaffected by dietary copper supplementation, although copper supplementation did reduce neonatal cartilage abnormalities. A potential explanation for these observations is that during the first month's post-partum, OCD is a very dynamic disease in which lesions appear and then may regress (Dik et al. 1999). Neonatal liver copper levels had no relationship with radiographic OCD changes, but significantly influenced the repair of these lesions in the period 5–11 mo post-partum (van Weeren et al., 2003). The authors suggested that copper is not instrumental for the aetiopathogenesis of OCD, but is important for the repair of the lesions and, hence, the number that will manifest themselves clinically. This may explain why previous experiments (Knight et al., 2003) involving supplementation of the mare and subsequently the foal could only find significant differences at 180 d in favor of the supplemented groups. It remains to be seen as to whether supplemented foals from deficient mares are able to use the available copper to effect repairs of the likely congenital lesions.

LINKING OBESITY AND JOINT DISEASE

It is clear that nutrition plays a substantial role in the normal physiological functioning of articular cartilage and that nutritional imbalance may contribute to the pathogenesis of OA and OCD and potentially, as discussed here, may play a role in the treatment of diseases, such as OA and OCD. In addition, nutrition, diet, and obesity are clearly related and may contribute to the progression of these diseases.

Obesity is a well documented clinical entity in human medicine, with prevalence estimated at 20.8% adults in Australia (Cameron et al., 2003) and 30.5% in the United States (Flegal et al., 2002). In veterinary practice, obesity is regularly encountered, although documented and diagnosed in its own right far less frequently (LaFlamme et al., 1994; Lund et al. 1999); the true prevalence is unknown, although estimates of approximately 25% of the dog and cat population in the U.S. have been suggested (Lund et al., 1999). This is hampered by the difficulty of accurately assessing and diagnosing this disease in domestic species.

Many diseases have been associated with obesity in cats and dogs, including those affecting the musculoskeletal systems (Mason, 1970; Hand et al., 1989), and it is accepted that overweight individuals are at an increased risk of osteoarthritis (Felson, 1995, 1996; Cicuttini et al., 1996, 1997). The role of obesity in the aetiology of OA is unclear, although there is a causal relationship in knee OA in humans. The results of a long-term study (Felson et al., 1988; Felson et al., 1992) have confirmed that obesity precedes the onset of OA. It may be that the increased mechanical forces acting on OA susceptible joints simply exacerbates pre-existing cartilage damage, but it is possible there may be systemic factors at work, since studies in humans have suggested a link between obesity and exacerbation of the signs of hand OA, where it is unlikely to be a significant increase in weight linked mechanical forces (Felson 1995; Felson and Zhang, 1998). However, evidence for this link is inconclusive, and a mechanism to explain this observation is not yet available.

In humans, obesity is a multifaceted disorder, whereas in domestic species, it is far more likely to result from an imbalance of calorific intake to expenditure. Strategies for tackling these problems are beyond the scope of this article, but studies have

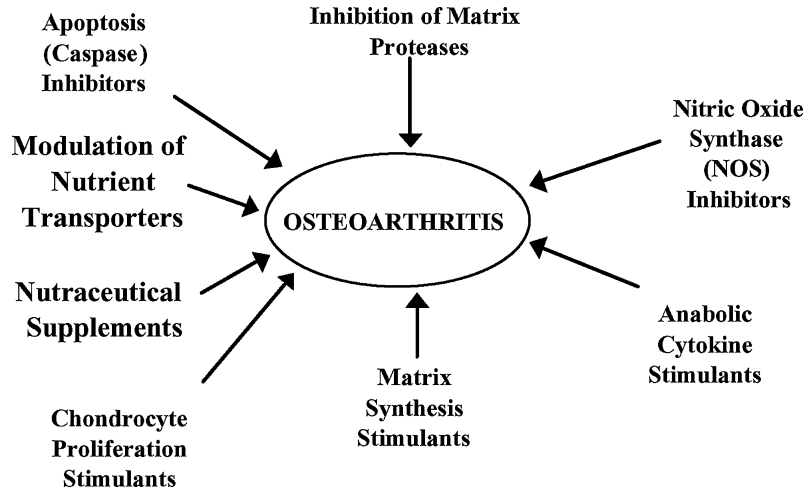

Figure 6 Rational therapies that may be used for the treatment of OA including nutraceutical supplementation and modulation of nutrient transporters. Inhibition of apoptotic pathways in OA cartilage and controlling exogenous nitric oxide levels are other potential therapeutic, strategies, which we have recently reviewed in detail (Mobasheri, 2002; Goggs et al., 2003).

confirmed that which was intuitively suggested, that weight loss through dietary or other means reduces the risk of development and progression of OA. The Framingham OA Study (Felson et al., 1988) suggests that loss of two body mass index (BMI) units, approximately 5 kg, reduces the risk of developing symptomatic knee OA in the following 10 yr by 50%. In dogs and cats, no studies have been performed that related obesity alone to the development of OA in non-predisposed joints. However, it has been confirmed that weight loss is beneficial to joints predisposed to OA, such as dysplastic hips (Kealy et al., 1992, 1997). Clearly we must elucidate further the links between diet, obesity, and joint diseases, and in the short-term, to design individually tailored programs for controlled weight loss to improve quality of life and improve the prognosis for disease.

CONCLUSION

It is clear from the literature, various meta- and mega-analyses, and this article that the subject of nutraceuticals is highly controversial and fraught with confusing and conflicting reports of efficacy, safety, and mechanisms of action. If one seeks to form a balanced opinion based on the current state of the literature, then it is vital that the methodology of any experimental publication is examined in detail, in order to uncover any potential bias or study compromise. Furthermore, when seeking to compare and combine results from reports, it is vital that we link studies alike in their methods. Numerous differences in methodology exist, from obvious differences between *in vivo* versus *in vitro* studies, to the concentrations of reagents used, the composition of culture media, and the type of culture, that is, 2- or 3-dimentional, alginate beads or explanted tissues, and the time course chosen. While we must try to extrapolate and compose a reasoned opinion based on all the available studies, it is important to realize that this may, in itself, be flawed.

This article also highlights the deficits in our knowledge and understanding of mechanisms of action of nutraceuticals and additionally illustrates that our knowledge of mechanisms involved in the uptake and metabolism of key nutrients and minerals in articular cartilage is insufficient. Chondrocytes exist in the unusually harsh, avascular environment of the ECM of articular cartilage. Essential nutrients, such as glucose, amino acids, and vitamins, vital for the function, growth, and development of cartilage, need to diffuse from the synovial microcirculation to the chondrocyte.Thus, it is essential to understand the mechanisms responsible for their transport. Developing therapies for degenerative joint disorders present exciting clinical challenges that rely upon a thorough understanding of pathogenesis and pathophysiology. The development of disease-modifying drugs, the quest for more effective pain relief, and most importantly, preventative measures must take into consideration the critical importance of nutritional factors. There are recent indications that investigators in the field are beginning to examine the role of diet, nutrient supplementation, and "metabolomics" in the management of OA and other degenerative joint disorders. A recent metabolic fingerprinting study in osteoarthritic guinea pigs suggests that nuclear magnetic resonance (NMR) spectroscopy may be used to obtain a metabolic fingerprint of biomarkers in urine to identify disease-specific profiles of urinary metabolites (Lamers et al., 2003). This approach has allowed for the detection of differences in OA-specific metabolites induced by different dietary vitamin C intakes, confirming the beneficial effects of vitamin C for treating osteoarthritic guinea pigs. This study has also shown that energy metabolism, particularly glucose and purine metabolism, is of major importance in OA.

In this article, we have discussed the role of nutrients and nutraceuticals in modulating the homeostasis of cartilage matrix and highlighted the importance of future expanded studies on plasma membrane transport systems responsible for their uptake by enterocytes in the small intestine and by chondrocytes and synoviocytes in articular cartilage and synovium, respectively. Given that membrane transporters are also targets of many drugs, future investigations aimed at developing therapeutic compounds should target nutrient transporters on the plasma membrane of chondrocytes and the signalling pathways responsible for their regulation (Figure 6). Nutraceuticals are currently commonly used for OA in humans and veterinary species. In most cases, the marketing of nutraceutical products has been extensive and often based on minimal experimental data; evidence for real efficacy is still lacking; therefore, large, controlled, double-blinded, randomized clinical studies are required to determine the effect of nutraceuticals on symptom and structure modification in OA. We have identified a number of key studies that must be undertaken in order to clarify the mechanisms by which nutraceuticals act and to determine the best way to use these potentially therapeutic compounds singly, in combination, or as adjuncts to NSAID therapy.

From the number of nutraceutical products in both the human and veterinary marketplaces, it is clear that these products are not only here to stay, but that they also have a number of end users. Overall, it appears that there is some evidence to support some of the claims for efficacy of nutraceuticals, but given the lack of extensive well-designed clinical studies, we must be cautious in advocating their widespread use. It is particularly inadvisable to compare these drugs too greatly to well-established therapeutic agents, such as NSAIDs, which have a substantial base of evidence both for their mechanism of action and for their clinical efficacy in joint disease. Perhaps, at present, their best place in the clinical armory is as adjuncts to conventional therapy, although patients should be chosen carefully, until the potential safety concerns regarding insulin resistance and immunosuppression are clarified.

The scientific community would welcome greater transparency and uniformity of commercial products, since many patients and nutraceutical consumers may be unaware of the variability of product constituents, such as glucosamine hydrochloride versus glucosamine sulphate and additionally in terms of the differences in the delivery system. Consumers must be able to

make a fully informed choice. We would also welcome a much greater regulation of these products by drug licensing bodies, in order to ensure uniformity and reliability of nutraceutical preparations. Studies in the United States have revealed that a number of preparations claiming to contain certain doses of glucosamine or chondroitin sulphate have significantly less (or none) of the dosages described (Deal and Moskowitz, 1999). In addition to the importance of this to consumers, it is essential that studies performed with these agents use preparations that are carefully defined in manufacture.

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