Nutraceutical Strategies for Preserving Cartilage in Osteoarthritis

Mark F. McCarty, NutriGuard Research, 1051 Hermes Ave., Encinitas, CA 92024

Abstract

The loss of cartilage associated with osteoarthritis (OA) appears to be largely attributable to a pro-catabolic effect – reflecting up-regulation of extracellular proteolytic activity – of interleukin-1 (IL-1) on chondrocytes. Cell culture studies in which chondrocytes are exposed to IL-1, as well as animal models of OA, can help to identify agents which have the potential to impede the signaling mechanisms whereby IL-1 evokes cartilage catabolism. Current evidence suggests that antioxidant strategies may be useful in this regard – notably, spirulina-derived phycocyanobilin, phase 2-inducer phytochemicals, and possibly N-acetylcysteine; this reflects an obligate role for NADPH oxidase activation in IL-1's catabolic impact. Effective activators of AMP-activated kinase such as berberine may likewise be useful, in part because of post-transcriptional down-regulation of iNOS expression. Glucosamine – arguably in higher doses than have been employed in most clinical trials – as well as vitamin D and fish oil, may also have chondroprotective potential. In light of suggestive evidence that metabolic syndrome may increase risk for OA – independent of the adverse impact of overweight on weight-bearing joints – lifestyle measures for preventing or reversing this syndrome may aid cartilage preservation. Hence, complex nutraceutical regimens and appropriate lifestyle strategies may have considerable potential for preventing early OA from progressing to a crippling condition.

Mechanisms of Cartilage Loss in Osteoarthritis

The cartilage loss associated with osteoarthritis (OA) is believed to reflect a catabolic impact of certain cytokines - most notably interleukin-1 (IL-1) – on chondrocytes; this IL-1 may stem from synovium, infiltrating leukocytes, or autocrine production.[1] IL-1 stimulates matrix degradation by boosting the transcription and synthesis of various metalloproteinases - including the collagenases MMP-1, -3, and -13 – and aggrecanases, while suppressing production of their functional antagonist tissue inhibitor of metalloproteinases (TIMP-1). Activation of NF-kappaB, AP-1, JNK, and p38 MAP kinase plays a key role in these effects. IL-1 also induces oxidative stress in chondrocytes via NADPH oxidase, while increasing expression of iNOS and of Cox-2; the former two effects contribute to the catabolic impact of IL-1 on cartilage matrix, as inhibitors of NADPH oxidase or of iNOS lessen this impact.[2-7] The role of Cox-2 and or prostanoids is more equivocal. In chondrocytes from mice deficient in microsomal prostaglandin E synthase activity, IL-1 is less effective for boosting MMP-3 and MMP-13 expression; this might reflect a catabolic role for prostaglandin receptor EP4. On the other hand, the prostaglandin receptor EP2 exerts an anti-catabolic impact on IL-1-treated chondrocytes, suggestive of a protective feedback mechanism (and possibly helping to explain the failure of NSAID therapy to prevent cartilage loss in OA).[8, 9] IL-1 exposure can also provoke cell death and suppress proteoglycan synthesis in chondrocytes; these effect requires joint production of superoxide and nitric oxide, and appear to be mediated by peroxynitrite.[10-13]

Antioxidant Strategies for Cartilage Preservation

Since induction of oxidative stress – via NOX2/NOX4-dependent NADPH oxidase activity – is a key mediator of the pro-catabolic signaling of IL-1 in chondrocytes, it is reasonable to suspect that effective antioxidant measures could be protective in this regard. There is recent evidence that free bilirubin, generated intracellularly by heme oxygenase activity, functions to inhibit various NAPDH oxidase complexes; moreover, the spirulina-derived phytochemical phycocyanobilin (PhyCB), a homologue and derivative of biliverdin, can mimic this effect.[14-18] Hence, ingestion of spirulina or of PhyCB-enriched spirulina extracts may have potential for preserving cartilage in OA. Although spirulina or PhyCB has not yet been examined in animal or cell culture models of OA, oral administration of spirulina has proven effective in rodent models of inflammatory arthritis.[19-21]

Phase 2 inducer phytochemicals, which promote expression of a range of antioxidant enzymes and boost glutathione synthesis,[22] might also be expected to counter the oxidative stress signaling required for IL-1's catabolic activity. Indeed, phase 2 inducers such as sulforaphane, epigallocatechin-3-gallate, chlorogenic acid, and diallyl sulfide have been reported to impede metalloproteinase induction or cell death in IL-1-stimulated chondrocytes.[23-29] Moreover, regular consumption of phase-2-inducing allium vegetables (garlic, onions) has been linked to decreased risk for OA of the hip in a cross-sectional epidemiological study.[30] The phase 2-inducible enzyme heme oxygenase-1 – as noted, a source of antioxidant bilirubin - may mediate some of these benefits; when induced with cobalt protoporphyrin IX in chondrocytes, this enzyme exerts both anabolic and anti-catabolic effects.[31] Surprisingly, N-acetylcysteine (NAC), which promotes glutathione synthesis, failed to modify the catabolic impact of IL-1 on chondrocytes in one study (albeit it did have this effect on IL-1-stimulated synoviocytes).[32] Yet other researchers reported that NAC protected chondrocytes from NO-induced apoptosis (an effect abrogated by an inhibitor of glutathione synthesis), and, when administered by intra-articular injection, diminished cartilage loss and chondrocyte apoptosis in a rat model of OA.[33]

With respect to the suspected key role of peroxynitrite in OA pathogenesis, it is interesting to note that reduced intracellular metabolites of folic acid (e.g. 5-methyltetrahydrofolate) have potent scavenging activity for peroxynitrite-derived antioxidants.[34] For this reason, in cells which concentrate folic acid against a gradient – such as vascular endothelial cells – high-dose folate can exert outstanding antioxidant activity.[35, 36] It would be therefore be interesting to determine whether folic acid could ameliorate the peroxynitrite-driven effects of IL-1 exposure on cultured chondrocytes.

AMPK Activators

AMP-activated protein kinase (AMPK) is expressed by chondrocytes, but a recent study shows that this expression is decreased in the articular chondrocytes of patients with knee OA.[37] In vitro, IL-1 exposure lessens AMPK activity in chondrocytes, and knock-down of AMPK in chondrocytes exacerbates the pro-catabolic impact of IL-1; conversely, activation of AMPK in chondrocytes antagonizes IL-1's catabolic activity.[37] The utility of metformin in diabetes reflects its ability to activate AMPK; unfortunately, metformin has not yet been studied in animal models or OA, nor has the impact of metformin on the progression of OA in diabetics been examined. However, the herb-derived agent berberine, long used to treat diabetes in China, is now known to activate AMPK in a manner comparable to metformin,[38-40] and recent studies suggest that it may have potential in the management of OA. In rat chondrocytes, berberine antagonized the impact of IL-1 on expression of MMP-1, MMP-3, MMP-13, and TIMP-1, and also

inhibited IL-1's impact on NO production.[41, 42] When OA was induced in rats by intra-articular injection of IL-1, concurrent injection of berberine exerted chondroprotective activity.[41] These considerations suggest that berberine and quite likely metformin may have some potential for aiding cartilage preservation in OA.

The molecular basis of AMPK's anti-catabolic impact on IL-1-stimulated chondrocytes remains unclear, but it is interesting to note that AMPK activity has suppressed iNOS induction in other types of cells exposed to endotoxin or pro-inflammatory cytokines.[43-47] This may reflect AMPK's ability to force nuclear sequestration of HuR, a protein which stabilizes iNOS mRNA.[48-51] It is reasonable to suspect that berberine and PhyCB could provide important complementary benefit in OA by suppressing excessive production of both NO and superoxide – thereby impeding IL-1 signal transduction while lessening peroxynitrite generation.[12]

Additional Options: Glucosamine, Vitamin D, Fish Oil

Oral administration of glucosamine has provided symptomatic relief and slowed loss of knee cartilage in some though not all clinical studies.[52-55] Glucosamine has shown clear chondroprotective activity in animal models of OA.[56-61] The inconsistent results of clinical studies with this agent may reflect that fact that the dose of glucosamine standardly used in these studies (1.5 g daily) fails to achieve the plasma levels of glucosamine observed in successful animal trials; hence, future clinical studies should evaluate higher intakes.[62] In chondrocytes stimulated with IL-1, glucosamine has been shown to suppress induced expression of the collagenase MMPs, while blocking activation of NF-kappaB and p38 MAP kinase.[63-70] Conceivably, glucosamine's impact in this regard reflects O-GlcNAc modification of IL-1 signaling intermediates, as this is the mechanism whereby glucosamine antagonizes the pro-inflammatory effect TNF-alpha on endothelial cells.[71] Moreover, in TNF-alpha-treated vascular smooth muscle cells incubated with glucosamine, O-GlcNAc modification of the p65 subunit of NF-kappaB blocks an activating phophorylation on Ser536 required for optimal transcriptional activity.[72]

In several studies, good vitamin D status has been associated with reduced risk for progression in OA of the knee; it is presumed that this reflects a favorable effect of vitamin D on the interaction of cartilage and subchondral bone.[73, 74] On the other hand, vitamin D status does not appear to influence the prevalence of knee OA – albeit one study found that knee OA was less common in people under 55 whose vitamin D status was good.[75]

Dietary fish oil has been found to confer dose-dependent symptomatic benefit in dogs with OA.[76, 77] In contrast, there is little evidence to-date that fish oil supplementation is useful in humans with OA. Perhaps this reflects the fact that the anti-inflammatory impact of omega-3 fats requires a substantial increase in tissue omega-3/omega-6 ratio, which may be hard to achieve in humans with high omega-6 intakes. In IL-1 exposed chondrocytes, EPA has shown anti-catabolic effects, whereas DHA is less effective in this regard.[78, 79] Likely, EPA opposes the production of certain arachidonate-derived prostanoids with pro-catabolic activity.

A Role for Metabolic Sydrome in OA Pathogenesis

Cross-sectional studies report that metabolic syndrome is more common in patients with OA.[80, 81] And patients with metabolic syndrome are more prone to OA of the hand than those without it – suggesting

that OA is not merely a consequence of overweight in metabolic syndrome, but rather may reflect the systemic inflammation associated with this syndrome.[82] Perhaps metabolic syndrome plays a role in triggering the cytokine production that drives OA. In Seventh Day Adventists, frequent consumption of meat (rich in saturated fats that are key mediators of metabolic syndrome[83, 84]) has been linked to increased prevalence of degenerative arthritis, even when body weight is taken into consideration in multivariate analysis.[85] Hence, dietary and lifestyle measures which help to prevent or reverse metabolic syndrome – with the exception of *high-impact* aerobic exercise! – might have some utility for preventing or ameliorating the course of OA (in addition to the inherent benefit of appropriate weight loss for prevention of OA in weight-bearing joints).

Summary

In conclusion, optimal nutraceutical and lifestyle strategies may have considerable scope for preventing cartilage loss in OA. Nutraceuticals of potential benefit in this regard include spirulina-derived PhyCB, phase 2-inducing phytochemicals, NAC, berberine, glucosamine, vitamin D, and fish oil. Whereas the impact of any one of these agents in isolation might be expected to be modest, combination regimens may prove to have greater utility; indeed, such regimens could be tested in chondrocyte cell cultures or in animal models of OA. Moreover, the agents under consideration here are likely to work in a number of ways to promote general health, and hence are recommendable even if their impact on OA proves modest.[18, 86-88] In this regard, metformin - which functions in a manner almost identical to berberine - has been linked to decreased cancer risk and lower overall mortality in diabetics (relative to other diabetes treatments), and may have potential as an "anti-aging" calorie restriction mimetic.[89-93] And, rather surprisingly, recent epidemiology has linked glucosamine use to decreased overall mortality and decreased risk for lung and colorectal cancer (possibly reflecting a broader anti-inflammatory role for this compound).[94-96]

References

(1) Pelletier JP, DiBattista JA, Roughley P, McCollum R, Martel-Pelletier J. Cytokines and inflammation in cartilage degradation. *Rheum Dis Clin North Am* 1993 August;19(3):545-568.

(2) Lo YY, Conquer JA, Grinstein S, Cruz TF. Interleukin-1 beta induction of c-fos and collagenase expression in articular chondrocytes: involvement of reactive oxygen species. *J Cell Biochem* 1998 April 1;69(1):19-29.

(3) Yasuhara R, Miyamoto Y, Akaike T, Akuta T, Nakamura M, Takami M, Morimura N, Yasu K, Kamijo R. Interleukin-1beta induces death in chondrocyte-like ATDC5 cells through mitochondrial dysfunction and energy depletion in a reactive nitrogen and oxygen species-dependent manner. *Biochem J* 2005 July 15;389(Pt 2):315-323.

(4) Grange L, Nguyen MV, Lardy B, Derouazi M, Campion Y, Trocme C, Paclet MH, Gaudin P, Morel F. NAD(P)H oxidase activity of Nox4 in chondrocytes is both inducible and involved in collagenase expression. *Antioxid Redox Signal* 2006 September;8(9-10):1485-1496.

(5) Ahmad R, Sylvester J, Ahmad M, Zafarullah M. Involvement of H-Ras and reactive oxygen species in proinflammatory cytokine-induced matrix metalloproteinase-13 expression in human articular chondrocytes. *Arch Biochem Biophys* 2011 March 15;507(2):350-355.

(6) Pelletier JP, Jovanovic D, Fernandes JC, Manning P, Connor JR, Currie MG, Di Battista JA, Martel-Pelletier J. Reduced progression of experimental osteoarthritis in vivo by selective inhibition of inducible nitric oxide synthase. *Arthritis Rheum* 1998 July;41(7):1275-1286.

(7) Pelletier JP, Lascau-Coman V, Jovanovic D, Fernandes JC, Manning P, Connor JR, Currie MG, Martel-Pelletier J. Selective inhibition of inducible nitric oxide synthase in experimental osteoarthritis is associated with reduction in tissue levels of catabolic factors. *J Rheumatol* 1999 September;26(9):2002-2014.

(8) Gosset M, Pigenet A, Salvat C, Berenbaum F, Jacques C. Inhibition of matrix metalloproteinase-3 and -13 synthesis induced by IL-1beta in chondrocytes from mice lacking microsomal prostaglandin E synthase-1. *J Immunol* 2010 November 15;185(10):6244-6252.

(9) Otsuka S, Aoyama T, Furu M, Ito K, Jin Y, Nasu A, Fukiage K, Kohno Y, Maruyama T, Kanaji T, Nishiura A, Sugihara H, Fujimura S, Otsuka T, Nakamura T, Toguchida J. PGE2 signal via EP2 receptors evoked by a selective agonist enhances regeneration of injured articular cartilage. *Osteoarthritis Cartilage* 2009 April;17(4):529-538.

(10) Yasuhara R, Miyamoto Y, Akaike T, Akuta T, Nakamura M, Takami M, Morimura N, Yasu K, Kamijo R. Interleukin-1beta induces death in chondrocyte-like ATDC5 cells through mitochondrial dysfunction and energy depletion in a reactive nitrogen and oxygen species-dependent manner. *Biochem J* 2005 July 15;389(Pt 2):315-323.

(11) Oh M, Fukuda K, Asada S, Yasuda Y, Tanaka S. Concurrent generation of nitric oxide and superoxide inhibits proteoglycan synthesis in bovine articular chondrocytes: involvement of peroxynitrite. *J Rheumatol* 1998 November;25(11):2169-2174.

(12) Jouzeau JY, Pacquelet S, Boileau C, Nedelec E, Presle N, Netter P, Terlain B. Nitric oxide (NO) and cartilage metabolism: NO effects are modulated by superoxide in response to IL-1. *Biorheology* 2002;39(1-2):201-214.

(13) Davies CM, Guilak F, Weinberg JB, Fermor B. Reactive nitrogen and oxygen species in interleukin-1-mediated DNA damage associated with osteoarthritis. *Osteoarthritis Cartilage* 2008 May;16(5):624-630.

(14) Lanone S, Bloc S, Foresti R, Almolki A, Taille C, Callebert J, Conti M, Goven D, Aubier M, Dureuil B, El-Benna J, Motterlini R, Boczkowski J. Bilirubin decreases nos2 expression via inhibition of NAD(P)H oxidase: implications for protection against endotoxic shock in rats. *FASEB J* 2005 November;19(13):1890-1892.

(15) Matsumoto H, Ishikawa K, Itabe H, Maruyama Y. Carbon monoxide and bilirubin from heme oxygenase-1 suppresses reactive oxygen species generation and plasminogen activator inhibitor-1 induction. *Mol Cell Biochem* 2006 October;291(1-2):21-28.

(16) Jiang F, Roberts SJ, Datla S, Dusting GJ. NO modulates NADPH oxidase function via heme oxygenase-1 in human endothelial cells. *Hypertension* 2006 November;48(5):950-957.

(17) Datla SR, Dusting GJ, Mori TA, Taylor CJ, Croft KD, Jiang F. Induction of heme oxygenase-1 in vivo suppresses NADPH oxidase derived oxidative stress. *Hypertension* 2007 October;50(4):636-642.

(18) McCarty MF. Clinical potential of Spirulina as a source of phycocyanobilin. *J Med Food* 2007 December;10(4):566-570.

(19) Remirez D, Gonzalez R, Merino N, Rodriguez S, Ancheta O. Inhibitory effects of Spirulina in zymosan-induced arthritis in mice. *Mediators Inflamm* 2002 April;11(2):75-79.

(20) Rasool M, Sabina EP, Lavanya B. Anti-inflammatory effect of Spirulina fusiformis on adjuvant-induced arthritis in mice. *Biol Pharm Bull* 2006 December;29(12):2483-2487.

(21) Kumar N, Singh S, Patro N, Patro I. Evaluation of protective efficacy of Spirulina platensis against collagen-induced arthritis in rats. *Inflammopharmacology* 2009 June;17(3):181-190.

(22) Jeong WS, Jun M, Kong AN. Nrf2: a potential molecular target for cancer chemoprevention by natural compounds. *Antioxid Redox Signal* 2006 January;8(1-2):99-106.

(23) Facchini A, Stanic I, Cetrullo S, Borzi RM, Filardo G, Flamigni F. Sulforaphane protects human chondrocytes against cell death induced by various stimuli. *J Cell Physiol* 2011 July;226(7):1771-1779.

(24) Ahmed S, Rahman A, Hasnain A, Lalonde M, Goldberg VM, Haqqi TM. Green tea polyphenol epigallocatechin-3-gallate inhibits the IL-1 beta-induced activity and expression of cyclooxygenase-2 and nitric oxide synthase-2 in human chondrocytes. *Free Radic Biol Med* 2002 October 15;33(8):1097-1105.

(25) Ahmed S, Wang N, Lalonde M, Goldberg VM, Haqqi TM. Green tea polyphenol epigallocatechin-3-gallate (EGCG) differentially inhibits interleukin-1 beta-induced expression of matrix metalloproteinase-1 and -13 in human chondrocytes. *J Pharmacol Exp Ther* 2004 February;308(2):767-773.

(26) Akhtar N, Haqqi TM. Epigallocatechin-3-gallate suppresses the global interleukin-1beta-induced inflammatory response in human chondrocytes. *Arthritis Res Ther* 2011 June 17;13(3):R93.

(27) Katiyar SK, Raman C. Green tea: a new option for the prevention or control of osteoarthritis. *Arthritis Res Ther* 2011 August 10;13(4):121.

(28) Chen WP, Tang JL, Bao JP, Hu PF, Shi ZL, Wu LD. Anti-arthritic effects of chlorogenic acid in interleukin-1beta-induced rabbit chondrocytes and a rabbit osteoarthritis model. *Int Immunopharmacol* 2011 January;11(1):23-28.

(29) Chen WP, Tang JL, Bao JP, Hu PF, Yu C, Shi ZL, Wu LD. Effects of diallyl sulphide in chondrocyte and cartilage in experimental osteoarthritis in rabbit. *Phytother Res* 2011 March;25(3):351-356.

(30) Williams FM, Skinner J, Spector TD, Cassidy A, Clark IM, Davidson RM, MacGregor AJ. Dietary garlic and hip osteoarthritis: evidence of a protective effect and putative mechanism of action. *BMC Musculoskelet Disord* 2010;11:280.

(31) Guillen M, Megias J, Gomar F, Alcaraz M. Haem oxygenase-1 regulates catabolic and anabolic processes in osteoarthritic chondrocytes. *J Pathol* 2008 March;214(4):515-522.

(32) Roman-Blas JA, Contreras-Blasco MA, Largo R, varez-Soria MA, Castaneda S, Herrero-Beaumont G. Differential effects of the antioxidant n-acetylcysteine on the production of catabolic mediators in IL-1beta-stimulated human osteoarthritic synoviocytes and chondrocytes. *Eur J Pharmacol* 2009 November 25;623(1-3):125-131.

(33) Nakagawa S, Arai Y, Mazda O, Kishida T, Takahashi KA, Sakao K, Saito M, Honjo K, Imanishi J, Kubo T. N-acetylcysteine prevents nitric oxide-induced chondrocyte apoptosis and cartilage degeneration in an experimental model of osteoarthritis. *J Orthop Res* 2010 February;28(2):156-163.

(34) Rezk BM, Haenen GR, Van d, V, Bast A. Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore. *FEBS Lett* 2003 December 18;555(3):601-605.

(35) Antoniades C, Shirodaria C, Warrick N, Cai S, de BJ, Lee J, Leeson P, Neubauer S, Ratnatunga C, Pillai R, Refsum H, Channon KM. 5-methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. *Circulation* 2006 September 12;114(11):1193-1201.

(36) McCarty MF, Barroso-Aranda J, Contreras F. High-dose folate and dietary purines promote scavenging of peroxynitrite-derived radicals--clinical potential in inflammatory disorders. *Med Hypotheses* 2009 November;73(5):824-834.

(37) Terkeltaub R, Yang B, Lotz M, Liu-Bryan R. Chondrocyte AMP-activated protein kinase activity suppresses matrix degradation responses to proinflammatory cytokines interleukin-1beta and tumor necrosis factor alpha. *Arthritis Rheum* 2011 July;63(7):1928-1937.

(38) Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Lee CH, Oh WK, Kim CT, Hohnen-Behrens C, Gosby A, Kraegen EW, James DE, Kim JB. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes* 2006 August;55(8):2256-2264.

(39) Turner N, Li JY, Gosby A, To SW, Cheng Z, Miyoshi H, Taketo MM, Cooney GJ, Kraegen EW, James DE, Hu LH, Li J, Ye JM. Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: a mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. *Diabetes* 2008 May;57(5):1414-1418.

(40) Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. *Metabolism* 2008 May;57(5):712-717.

(41) Hu PF, Chen WP, Tang JL, Bao JP, Wu LD. Protective effects of berberine in an experimental rat osteoarthritis model. *Phytother Res* 2011 June;25(6):878-885.

(42) Moon PD, Jeong HS, Chun CS, Kim HM. Baekjeolyusin-tang and its active component berberine block the release of collagen and proteoglycan from IL-1beta-stimulated rabbit

cartilage and down-regulate matrix metalloproteinases in rabbit chondrocytes. *Phytother Res* 2011 June;25(6):844-850.

(43) Pilon G, Dallaire P, Marette A. Inhibition of inducible nitric-oxide synthase by activators of AMP-activated protein kinase: a new mechanism of action of insulin-sensitizing drugs. *J Biol Chem* 2004 May 14;279(20):20767-20774.

(44) Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HJ, Kim WS, Kim JB. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. *Am J Physiol Endocrinol Metab* 2009 April;296(4):E955-E964.

(45) Peairs A, Radjavi A, Davis S, Li L, Ahmed A, Giri S, Reilly CM. Activation of AMPK inhibits inflammation in MRL/lpr mouse mesangial cells. *Clin Exp Immunol* 2009 June;156(3):542-551.

(46) Lu DY, Tang CH, Chen YH, Wei IH. Berberine suppresses neuroinflammatory responses through AMP-activated protein kinase activation in BV-2 microglia. *J Cell Biochem* 2010 June 1;110(3):697-705.

(47) Centeno-Baez C, Dallaire P, Marette A. Resveratrol inhibition of inducible nitric oxide synthase in skeletal muscle involves AMPK but not SIRT1. *Am J Physiol Endocrinol Metab* 2011 August 2.

(48) Wang W, Fan J, Yang X, Furer-Galban S, Lopez dS, I, von KC, Guo J, Georas SN, Foufelle F, Hardie DG, Carling D, Gorospe M. AMP-activated kinase regulates cytoplasmic HuR. *Mol Cell Biol* 2002 May;22(10):3425-3436.

(49) Wang W, Yang X, Kawai T, Lopez dS, I, Mazan-Mamczarz K, Chen P, Chook YM, Quensel C, Kohler M, Gorospe M. AMP-activated protein kinase-regulated phosphorylation and acetylation of importin alpha1: involvement in the nuclear import of RNA-binding protein HuR. *J Biol Chem* 2004 November 12;279(46):48376-48388.

(50) Di MS, Mazroui R, Dallaire P, Chittur S, Tenenbaum SA, Radzioch D, Marette A, Gallouzi IE. NF-kappa B-mediated MyoD decay during muscle wasting requires nitric oxide synthase mRNA stabilization, HuR protein, and nitric oxide release. *Mol Cell Biol* 2005 August;25(15):6533-6545.

(51) Pautz A, Art J, Hahn S, Nowag S, Voss C, Kleinert H. Regulation of the expression of inducible nitric oxide synthase. *Nitric Oxide* 2010 September 15;23(2):75-93.

(52) Bruyere O, Pavelka K, Rovati LC, Deroisy R, Olejarova M, Gatterova J, Giacovelli G, Reginster JY. Glucosamine sulfate reduces osteoarthritis progression in postmenopausal women with knee osteoarthritis: evidence from two 3-year studies. *Menopause* 2004 March;11(2):138-143.

(53) Bruyere O, Pavelka K, Rovati LC, Gatterova J, Giacovelli G, Olejarova M, Deroisy R, Reginster JY. Total joint replacement after glucosamine sulphate treatment in knee osteoarthritis: results of a mean 8-year observation of patients from two previous 3-year, randomised, placebo-controlled trials. *Osteoarthritis Cartilage* 2008 February;16(2):254-260.

(54) Wandel S, Juni P, Tendal B, Nuesch E, Villiger PM, Welton NJ, Reichenbach S, Trelle S. Effects of glucosamine, chondroitin, or placebo in patients with osteoarthritis of hip or knee: network meta-analysis. *BMJ* 2010;341:c4675.

(55) Vlad SC, LaValley MP, McAlindon TE, Felson DT. Glucosamine for pain in osteoarthritis: why do trial results differ? *Arthritis Rheum* 2007 July;56(7):2267-2277.

(56) Tiraloche G, Girard C, Chouinard L, Sampalis J, Moquin L, Ionescu M, Reiner A, Poole AR, Laverty S. Effect of oral glucosamine on cartilage degradation in a rabbit model of osteoarthritis. *Arthritis Rheum* 2005 April;52(4):1118-1128.

(57) Wang SX, Laverty S, Dumitriu M, Plaas A, Grynpas MD. The effects of glucosamine hydrochloride on subchondral bone changes in an animal model of osteoarthritis. *Arthritis Rheum* 2007 May;56(5):1537-1548.

(58) Scotto dA, Corsi A, Grillo MG, Cicione C, Calamia V, Panzini G, Sansone A, Giordano C, Politi L, Scandurra R. Effects of intra-articular administration of glucosamine and a peptidyl-glucosamine derivative in a rabbit model of experimental osteoarthritis: a pilot study. *Rheumatol Int* 2008 March;28(5):437-443.

(59) Lu F, Guo H. [Study on the role of glucosamine hydrochloride in the pathogenesis of osteoarthritis]. *Wei Sheng Yan Jiu* 2003 November;32(6):594-597.

(60) Naito K, Watari T, Furuhata A, Yomogida S, Sakamoto K, Kurosawa H, Kaneko K, Nagaoka I. Evaluation of the effect of glucosamine on an experimental rat osteoarthritis model. *Life Sci* 2010 March 27;86(13-14):538-543.

(61) Wen ZH, Tang CC, Chang YC, Huang SY, Hsieh SP, Lee CH, Huang GS, Ng HF, Neoh CA, Hsieh CS, Chen WF, Jean YH. Glucosamine sulfate reduces experimental osteoarthritis and nociception in rats: association with changes of mitogen-activated protein kinase in chondrocytes. *Osteoarthritis Cartilage* 2010 September;18(9):1192-1202.

(62) ghazadeh-Habashi A, Jamali F. The glucosamine controversy; a pharmacokinetic issue. *J Pharm Pharm Sci* 2011;14(2):264-273.

(63) Sandy JD, Gamett D, Thompson V, Verscharen C. Chondrocyte-mediated catabolism of aggrecan: aggrecanase-dependent cleavage induced by interleukin-1 or retinoic acid can be inhibited by glucosamine. *Biochem J* 1998 October 1;335 (Pt 1):59-66.

(64) Gouze JN, Bordji K, Gulberti S, Terlain B, Netter P, Magdalou J, Fournel-Gigleux S, Ouzzine M. Interleukin-1beta down-regulates the expression of glucuronosyltransferase I, a key enzyme priming glycosaminoglycan biosynthesis: influence of glucosamine on interleukin-1beta-mediated effects in rat chondrocytes. *Arthritis Rheum* 2001 February;44(2):351-360.

(65) Gouze JN, Bianchi A, Becuwe P, Dauca M, Netter P, Magdalou J, Terlain B, Bordji K. Glucosamine modulates IL-1-induced activation of rat chondrocytes at a receptor level, and by inhibiting the NF-kappa B pathway. *FEBS Lett* 2002 January 16;510(3):166-170.

(66) Largo R, varez-Soria MA, ez-Ortego I, Calvo E, Sanchez-Pernaute O, Egido J, Herrero-Beaumont G. Glucosamine inhibits IL-1beta-induced NFkappaB activation in human osteoarthritic chondrocytes. *Osteoarthritis Cartilage* 2003 April;11(4):290-298.

(67) Nakamura H, Shibakawa A, Tanaka M, Kato T, Nishioka K. Effects of glucosamine hydrochloride on the production of prostaglandin E2, nitric oxide and metalloproteases by chondrocytes and synoviocytes in osteoarthritis. *Clin Exp Rheumatol* 2004 May;22(3):293-299.

(68) Gouze JN, Gouze E, Popp MP, Bush ML, Dacanay EA, Kay JD, Levings PP, Patel KR, Saran JP, Watson RS, Ghivizzani SC. Exogenous glucosamine globally protects chondrocytes from the arthritogenic effects of IL-1beta. *Arthritis Res Ther* 2006;8(6):R173.

(69) Valvason C, Musacchio E, Pozzuoli A, Ramonda R, Aldegheri R, Punzi L. Influence of glucosamine sulphate on oxidative stress in human osteoarthritic chondrocytes: effects on HO-1, p22(Phox) and iNOS expression. *Rheumatology (Oxford)* 2008 January;47(1):31-35.

(70) Byron CR, Stewart MC, Stewart AA, Pondenis HC. Effects of clinically relevant concentrations of glucosamine on equine chondrocytes and synoviocytes in vitro. *Am J Vet Res* 2008 September;69(9):1129-1134.

(71) Ju Y, Hua J, Sakamoto K, Ogawa H, Nagaoka I. Modulation of TNF-alpha-induced endothelial cell activation by glucosamine, a naturally occurring amino monosaccharide. *Int J Mol Med* 2008 December;22(6):809-815.

(72) Xing D, Gong K, Feng W, Nozell SE, Chen YF, Chatham JC, Oparil S. O-GlcNAc modification of NFkappaB p65 inhibits TNF-alpha-induced inflammatory mediator expression in rat aortic smooth muscle cells. *PLoS ONE* 2011;6(8):e24021.

(73) McAlindon TE, Felson DT, Zhang Y, Hannan MT, Aliabadi P, Weissman B, Rush D, Wilson PW, Jacques P. Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. *Ann Intern Med* 1996 September 1;125(5):353-359.

(74) Bergink AP, Uitterlinden AG, van Leeuwen JP, Buurman CJ, Hofman A, Verhaar JA, Pols HA. Vitamin D status, bone mineral density, and the development of radiographic osteoarthritis of the knee: The Rotterdam Study. *J Clin Rheumatol* 2009 August;15(5):230-237.

(75) Heidari B, Heidari P, Hajian-Tilaki K. Association between serum vitamin D deficiency and knee osteoarthritis. *Int Orthop* 2010 December 30.

(76) Fritsch D, Allen TA, Dodd CE, Jewell DE, Sixby KA, Leventhal PS, Hahn KA. Dose-titration effects of fish oil in osteoarthritic dogs. *J Vet Intern Med* 2010 September;24(5):1020-1026.

(77) Fritsch DA, Allen TA, Dodd CE, Jewell DE, Sixby KA, Leventhal PS, Brejda J, Hahn KA. A multicenter study of the effect of dietary supplementation with fish oil omega-3 fatty acids on carprofen dosage in dogs with osteoarthritis. *J Am Vet Med Assoc* 2010 March 1;236(5):535-539.

(78) Wann AK, Mistry J, Blain EJ, Michael-Titus AT, Knight MM. Eicosapentaenoic acid and docosahexaenoic acid reduce interleukin-1beta-mediated cartilage degradation. *Arthritis Res Ther* 2010;12(6):R207.

(79) Zainal Z, Longman AJ, Hurst S, Duggan K, Caterson B, Hughes CE, Harwood JL. Relative efficacies of omega-3 polyunsaturated fatty acids in reducing expression of key proteins in a model system for studying osteoarthritis. *Osteoarthritis Cartilage* 2009 July;17(7):896-905.

(80) Puenpatom RA, Victor TW. Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgrad Med* 2009 November;121(6):9-20.

(81) Yoshimura N, Muraki S, Oka H, Kawaguchi H, Nakamura K, Akune T. Association of knee osteoarthritis with the accumulation of metabolic risk factors such as overweight, hypertension, dyslipidemia, and impaired glucose tolerance in Japanese men and women: the ROAD study. *J Rheumatol* 2011 May;38(5):921-930.

(82) Katz JD, Agrawal S, Velasquez M. Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Opin Rheumatol* 2010 September;22(5):512-519.

(83) Riccardi G, Giacco R, Rivellese AA. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin Nutr* 2004 August;23(4):447-456.

(84) McCarty MF. Dietary saturate/unsaturate ratio as a determinant of adiposity. *Med Hypotheses* 2010 July;75(1):14-16.

(85) Hailu A, Knutsen SF, Fraser GE. Associations between meat consumption and the prevalence of degenerative arthritis and soft tissue disorders in the adventist health study, California U.S.A. *J Nutr Health Aging* 2006 January;10(1):7-14.

(86) Giudice A, Montella M. Activation of the Nrf2-ARE signaling pathway: a promising strategy in cancer prevention. *Bioessays* 2006 February;28(2):169-181.

(87) Grant WB. An estimate of the global reduction in mortality rates through doubling vitamin D levels. *Eur J Clin Nutr* 2011 September;65(9):1016-1026.

(88) Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)* 2008 June;233(6):674-688.

(89) Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, Gandini S. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. *Cancer Prev Res (Phila)* 2010 November;3(11):1451-1461.

(90) Roussel R, Travert F, Pasquet B, Wilson PW, Smith SC, Jr., Goto S, Ravaud P, Marre M, Porath A, Bhatt DL, Steg PG. Metformin use and mortality among patients with diabetes and atherothrombosis. *Arch Intern Med* 2010 November 22;170(21):1892-1899.

(91) McCarty MF. Chronic activation of AMP-activated kinase as a strategy for slowing aging. *Med Hypotheses* 2004;63(2):334-339.

(92) Spindler SR, Mote PL. Screening candidate longevity therapeutics using gene-expression arrays. *Gerontology* 2007;53(5):306-321.

(93) Anisimov VN. Metformin for aging and cancer prevention. *Aging (Albany NY)* 2010 November;2(11):760-774.

(94) Pocobelli G, Kristal AR, Patterson RE, Potter JD, Lampe JW, Kolar A, Evans I, White E. Total mortality risk in relation to use of less-common dietary supplements. *Am J Clin Nutr* 2010 June;91(6):1791-1800.

(95) Satia JA, Littman A, Slatore CG, Galanko JA, White E. Associations of herbal and specialty supplements with lung and colorectal cancer risk in the VITamins and Lifestyle study. *Cancer Epidemiol Biomarkers Prev* 2009 May;18(5):1419-1428.

(96) Brasky TM, Lampe JW, Slatore CG, White E. Use of glucosamine and chondroitin and lung cancer risk in the VITamins And Lifestyle (VITAL) cohort. *Cancer Causes Control* 2011 September;22(9):1333-1342.