# A Key Role for Activation of Chondrocyte NADPH Oxidase in the Loss of Cartilage Matrix Associated with Osteoarthritis

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## Abstract

Cytokines, particular IL-1beta, are elevated in osteoarthritic cartilage, and are believed to mediate the progressive loss of cartilage matrix and of chondrocytes often associated with osteoarthritis (OA). IL-1 promotes a catabolic phenotype in chondrocytes, increasing the expression of collagen-degrading metalloproteinases and of aggrecanase, while decreasing proteoglycan synthesis and promoting chondrocyte apoptosis. These and other effects of IL-1 on chondrocyte metabolism appear to be contingent on increased chondrocyte superoxide generation, consequent to activation of NADPH oxidase. Hence, the spirulina-derived chromophore phycocyanobilin (PCB), recently shown to inhibit NADPH oxidase, may have potential for preserving cartilage mass in OA. Glucosamine, which antagonizes the catabolic impact of IL-1 on chondrocytes, has already demonstrated clinical efficacy in this regard. Melatonin may also help to preserve cartilage matrix, via induction of antioxidant enzymes and an increase in chondrocyte expression of TGF-beta. Thus, a supplementation regimen incorporating effective amounts of PCB, glucosamine, and melatonin may have a favorable long-term impact on joint health. These measures may also have potential for slowing the degeneration of intervertebral discs, a major cause of back pain and disability in the elderly.

### NADPH Oxidase as a Mediator of Cartilage Catabolism

Studies demonstrate that chondrocytes obtained from joints afflicted with osteoarthritis (OA) are under increased oxidant stress; in particular, an increase in lipid peroxidation products has been noted in these cells.[1-3] This is not surprising, inasmuch as both IL-1 and TNF-alpha, cytokines thought to be key mediators of the cartilage matrix degradation associated with OA,[4;5] stimulate superoxide production in chondrocytes.[6-10] The primary source of this superoxide appears to be NADPH oxidase, inasmuch as DPI and apocynin inhibit its production, and NADPH oxidase activity has been demonstrated in chondrocytes.[11-17] Reduced chondrocyte expression of certain antioxidant enzymes may exacerbate the oxidative stress associated with OA.[18]

Furthermore, there is evidence that this induced production of superoxide, sometimes in conjunction with iNOS-mediated production of NO, plays an obligate role in many processes that mediate the catabolic effects of IL-1/TNF-alpha on cartilage: increased c-fos expression, AP-1 activation, JNK activation, increased expression of MMPs with collagenase activity, NF-kappaB activation, increased cox-2 expression, increased iNOS expression, decreased proteoglycan synthesis, and chondrocyte apoptosis.[10;13;14;17;19-21] In addition, oxidants mediate the increased aggrecan degradation observed in LPS-treated chondrocyte cultures.[22] The net effect of these processes is an increase in cartilage

matrix degradation (primarily, proteolytic cleavage of collagen and aggrecan) that cannot be matched by a compensatory evoked increase in matrix synthesis – resulting in progressive loss of matrix mass. This process can be exacerbated by apoptotic loss of chondrocytes.[23]

These considerations suggest that long-term supplementation with phycocyanobilin (PCB), a chromophore found in spirulina and other cyanobacteria, could have a favorable effect on retention of cartilage matrix as people age. There is recent evidence that PCB, presumably after conversion to phycocyanorubin by the ubiquitously expressed enzyme biliverdin reductase, acts as a potent inhibitor of NADPH oxidase, mimicking the physiological activity of bilirubin in this regard (Inoguchi T, personal communication). Thus, supplemental PCB may have considerable potential for prevention or treatment of the many disorders in which oxidant stress attributable to NADPH oxidase up-regulation plays a pathogenic role.

With respect to the possibility of ingesting spirulina as a source of PCB, it should be noted that osteoarthritis chondrocytes express the TLR2 receptor, stimulation of which promotes a catabolic phenotype in chondrocytes.[24] Polysaccharides from spirulina have been reported to act as TLR2 agonists, and oral administration of these polysaccharides in mice has an immunomodulatory impact.[25] Thus, it is conceivable that isolated PCB will work more effectively than whole spirulina in preserving cartilage matrix. However, if the immune effects of orally administered spirulina polysaccharides are mediated primarily in the intestinal mucosa – as seems likely given the high molecular weight of these compounds – it may be difficult to achieve physiologically meaningful levels of these polysaccharides in cartilage, a relatively avascular tissue.

**Glucosamine and Niacinamide as an IL-1 Antagonists**

Furthermore, there is now considerable evidence that physiologically achievable concentrations of glucosamine also antagonize the pro-catabolic effects of IL-1 on chondrocytes[26-35] – a phenomenon which could explain the favorable impact of glucosamine supplementation on the progressive loss of cartilage mass associated with OA.[36-39] It would be of interest to test the joint impact of glucosamine and PCB on IL-1-stimulated chondrocytes. Concurrent supplementation with glucosamine and PCB might prove to be a feasible and well-tolerated strategy for slowing the loss of cartilage mass associated with OA and aging.

The basis of the analgesic impact of glucosamine therapy in OA is still unclear, and it remains to be seen whether PCB supplementation will have any clinical utility in this regard.

There is limited clinical evidence that high-dose supplemental niacinamide (approximately 3g daily in divided doses) may be useful in the management of OA.[40;41] The possibility that niacinamide, like glucosamine, might act as an antagonist of IL-1's impact on chondrocytes, has been raised.[41] Very recently, niacinamide has been shown to aid preservation of collagen and block induction of iNOS in rabbit annulus fibrosus (in

which chondrocytes are the sole cell type) exposed to IL-1.[42] Thus, the potential impact of high-dose niacinamide on cartilage integrity merits further study.

**Possible Relevance to Disc Degeneration**

These considerations might also be germane to prevention of intervertebral disc degeneration, a major cause of lower back pain and disability, particularly among the elderly. Recent studies show that chondrocytes in the nucleus pulposus and inner annular fibrosus express IL-1beta, and this expression tends to be increased in chondrocytes derived from degenerate discs.[43] Moreover, disc chondrocytes are IL-1 responsive in vitro, adopting a more catabolic phenotype analogous to that seen in articular chondrocytes exposed to this cytokine.[43;44] Of particular interest is the finding that, in chondrocytes from degenerative discs, exposure to exogenous IL-1 results in increased production of endogenous IL-1beta – whereas IL-1 treatment tends to suppress IL-1beta expression in chondrocytes from healthy discs; the implication is that, in degenerating discs, elevated IL-1 exposure is maintained by a positive feedback loop.[43;45] Although the NADPH oxidase activity of disc chondrocytes has not been studied, degenerating discs do appear to be under increased oxidative stress, as indicated by increased levels of the prominent advanced glycation end-product N-(carboxymethyl)lysine.[46;47] Thus, it seems not unlikely that IL-1-evoked oxidative stress, presumably attributable to NADPH oxidase, is a key mediator of the catabolic activity of chondrocytes in degenerating discs, as it is in articular chondrocytes exposed to IL-1.

In regard to the possible utility of glucosamine for preventing disc degeneration, a case report has described partial restoration of disc structure (increased disc water content verified by MRI) in a patient with lumbar disc degeneration treated for two years with glucosamine plus chondroitin sulfate.[48] It would be worthwhile to assess the impact of exogenous glucosamine on disc chondrocytes exposed to IL-1, in analogy to studies focusing on articular chondrocytes.

**Adjunctive Antioxidant Strategies**

While inhibition of NADPH oxidase represents a straightforward approach to controlling oxidant stress in chondrocytes, the adjunctive strategy of promoting endogenous antioxidant mechanisms of chondrocytes also merits consideration. Melatonin, acting via receptors, is known to enhance the expression and activity of a range of antioxidant enzymes in many tissues;[49;50] unfortunately, its possible impact on chondrocytes has received little study. However, a recent report concludes that exogenous melatonin has a restorative effect in rats with surgically-induced intervertebral disc degeneration.[51] Melatonin treatment was associated with increased expression of TGF-beta in disc chondrocytes; this hormone has an anabolic effect on chondroctyes antagonistic to IL-1 activity, and there is evidence that the chondrocytes of aging mice express less TGF-beta, and are less responsive to it.[52-54] Whether the antioxidant activity of melatonin plays any role in its effect on chondrocyte TGF-beta expression remains unclear. Nocturnal melatonin production is compromised by night shift work,[55] which has been linked to increased risk for disc degeneration in a prospective epidemiological study.[56] In

chickens, pinealectomy is associated with accelerated degeneration of intervertebral discs.[57] Nocturnal supplemental melatonin presumably would provide its greatest benefit in subjects who are middle-aged or elderly, as pineal melatonin production tends to decrease with increasing age.

Selenium is an essential component of several antioxidant enzymes (the various isoforms of glutathione peroxidases as well as thioredoxin reductase),[58] and in many regions typical diets do not supply enough selenium to optimize production of these enzymes.[59] It is therefore logical to expect that insuring optimal selenium status via supplementation or food enrichment could have a long-term beneficial impact on cartilage integrity. There do not appear to be any studies examining the impact of prolonged suboptimal selenium intakes on risk for osteoarthritis or cartilage loss; however, profound selenium deficiency during childhood is known to induce cartilage deformities – Kashin-Beck disease, a condition encountered in selenium-deficient regions of China.[60]

In a prospective study conducted in Boston, relatively low dietary intakes of vitamin C were linked to increased risk for cartilage loss and knee pain, but not to incident OA overall.[61] Less consistent trends suggested that vitamin E and beta carotene nutrition might also have some impact in this regard.

**Overview**

In summary, there is substantial evidence that activation of NADPH oxidase in chondrocytes of osteoarthritic cartilage – mediated by IL-1 and possibly other cytokines – is a key mediator of the progressive cartilage degradation that often accompanies OA. Supplemental PCB – either in spirulina or as an isolated nutraceutical – can inhibit NAPDH oxidase, and thus may have potential for promoting retention or regeneration of cartilage matrix. The catabolic effects of IL-1 on cartilage can also be opposed by physiologically achievable concentrations of glucosamine – presumably explaining why this nutraceutical has beneficial structure-modifying activity in OA. Nocturnal melatonin supplementation may also have potential in this regard, owing to melatonin's ability to induce antioxidant enzymes and promote TGF-beta expression in chondrocytes. Insuring adequate dietary intakes of selenium, vitamin C, and perhaps other nutritional antioxidants may also be beneficial to cartilage health. These measures may have potential not only for preserving the cartilage of articular joints, but also for preventing intervertebral disc degeneration.

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