Metabolic Mechanisms in the Pathogenesis of Osteoarthritis

A Review

Bruce P. Burnett, PhD
Robert Levy, MD
Brian J. Cole, MD, MBA

INTRODUCTION

Osteoarthritis is the major chronic disease leading to musculoskeletal morbidity and functional loss, its effects increasing with age. It is estimated that by 2020 osteoarthritis will be the fourth leading cause of disability in the United States.[52,89] Currently, approximately 20 million adults suffer from osteoarthritis, costing the US economy almost $60 billion per anum. The number of individuals affected is expected to rise to over 40 million in the next 25 years.[12,48] Despite a substantial amount of scientific and clinical research, there is no uniform agreement regarding the etiology and pathogenesis of the disease. It is generally agreed that causation is multifactorial, involving genetic predetermination, acute and chronic joint trauma, as well as metabolic and inflammatory mechanisms and, perhaps, dietary factors. This report is devoted to a review of the metabolic factors that lead directly and indirectly via inflammatory mediators to the cartilage damage characteristic of osteoarthritis.

CAUSES OF OSTEOARTHRITIS

Osteoarthritis has primarily been associated with aging.[27] The disease is usually initiated by damage as a result of injury or continued chronic overuse and mechanical strain on the joints.[15,25,26,53] Young adults who suffer acute trauma that causes ligament or meniscal tears have a higher incidence of osteoarthritis.[72] Muscle weakness from injury or degeneration associated with aging also results in higher incidences of osteoarthritis, suggesting that the musculature stabilizes the joint for proper alignment thereby lessening the load on articular cartilage.[23,41,67] Initial damage to the joint can lead to generalized pain, which restricts range of motion, musculature use, and may or may not be seen by radiographic examination in the early stages of the disease.[68] Progression of the disease state with age is characterized by structural degradation of the cartilage, which can be visualized microscopically and via radiographic analysis.[16,19,20,75] Initial degradation is characterized by a process called "fibrillation" or splitting of tissue (for review see Stockwell[77]). The cartilage becomes rough, death of superficial cells occurs, and the matrix immediately below the surface becomes impregnated with negatively charged proteins, different from proteins normally found in the cartilage. Proteoglycan destruction ensues causing a loss of the aggrecan matrix, which worsens over time. Although the initial damage is caused by injury and structural changes, other factors such as genetics, obesity, diet, and chronic inflammation lead to progression of the disease state.

Evidence suggests genetic factors may also contribute to the cause of osteoarthritis.[9] Studies of familial clusters of patients with osteoarthritis point to several different genes such as the collagen forming genes COL1A1 and COL2A1,[45,51,87] estrogen receptor-α, especially in postmenopausal women,[74,82] vitamin D receptor,[46,81] transforming growth factor β1 (TGFβ1),[91] insulin-like growth factor (IGF1),[63] and the gene for aggrecan proteoglycan.[42] All of these genes are influenced by environmental factors associated with aging. For example, dietary intake of vitamin D and calcium as well as the ability to process vitamins and minerals decreases with age. In postmenopausal women, decreased estrogen levels are associated with increased osteoarthritis.[43] Obesity, whether genetically predetermined or not, is the single-most contributing factor in the development of osteoarthritis.

Drs Burnett and Levy are from Primus Pharmaceuticals, Inc, Scottsdale, Ariz; and Dr Cole is from the Department of Orthopedics and Anatomy and Cell Biology and the Cartilage Restoration Center at Rush, Rush University Medical Center, Chicago, Ill.

Reprint requests: Brian J. Cole, MD, MBA, Dept of Orthopedics and Anatomy and Cell Biology, Rush University Medical Center, 1725 W Harrison, Ste 1063, Chicago, IL 60612.

The prevalence of osteoarthritis of the knee in obese individuals is about double that of normal-weight persons.[37,86] Conversely, weight loss reduces the risk of developing progressive osteoarthritis.[28] Good correlation exists between body mass index and osteoarthritis of the knee in women, but in men, the relationship is less clear.[2,29,36,58,59] Results from the Framingham Knee Osteoarthritis Study suggest that weight loss of approximately 5 kg reduces the risk for the development of knee osteoarthritis by 50% over the subsequent 10 years.[28] Hip and hand osteoarthritis also occur in obese persons at varying rates.[24] However, being overweight does not necessarily increase load across joints in the hand, suggesting involvement of other factors. There is increasing evidence that systemic factors such as chronic inflammation or other metabolic processes are involved in development or progression of osteoarthritis.[57] Recently, obesity was shown to produce chronic inflammation by increasing plasma concentrations of C-reactive protein, interleukin-6 (IL-6), and plasminogen activator inhibitor.[13] Not only does obesity put more stress on joints, but chronic inflammation leads to further metabolic and oxidative stress, which in turn contributes to the chemical signals that initiate osteoarthritis. This contribution of obesity and other behavioral factors in osteoarthritis initiation and progression suggests that dietary modification may help manage certain metabolic processes of the disease.

Dietary habits have been shown to specifically influence the metabolic and inflammatory processes involved in osteoarthritis.[47] Lower intake of vitamins C, D, and E are associated with increased incidence of osteoarthritis even in individuals who have not experienced joint trauma.[22] Increased intake of omega-3 polyunsaturated fatty acid from fish decreases degenerative and inflammatory chondrocyte metabolism in osteoarthritis.[17] Elderly patients in the United States, in particular those with osteoarthritis, suffer from excess intake of omega-6 polyunsaturated fatty acid in their diet, which is associated with higher levels of osteoarthritis.[93] Poor intake of antioxidants in conjunction with oxidative stress is also associated with chronic disease states in the elderly, a key factor in the etiology of osteoarthritis.[6,65] Conversely, intake of antioxidants improves osteoarthritis symptoms.[60] Finally, intake of flavonoids, low molecular weight compounds that are part of the larger class of compounds known as polyphenols, ubiquitous in plants, is associated with decreased incidence of chronic diseases.[64] Populations that have a lower consumption of flavonoid compounds derived from vegetable matter and a greater intake of omega-6 polyunsaturated fatty acid plus simple sugars, as in the US diet, have a higher incidence of osteoarthritis compared to other parts of the world.[64,78,93] These observations argue that dietary habits contribute to the metabolic and inflammatory etiology of osteoarthritis and that changes in diet may affect osteoarthritis progression.

All of the above factors—aging, obesity, overuse, acute trauma, muscle weakness, and diet—are potential catalysts for the pain and inflammation that characterize the osteoarthritic process. On a metabolic level, an essential change occurs in the joint leading to an "inflammatory cascade," which triggers the chronic overproduction of factors that influence the disease state. When expressed over years of time, these factors can worsen osteoarthritis and ultimately lead to disability.

INFLAMMATORY CASCADE

Osteoarthritis is characterized by progressive, degenerative changes of the synovial joint leading to a loss of hyaline articular cartilage, alterations in the subchondral bone, and marginal osteophyte formation.[77] The structure of hyaline cartilage consists primarily of water, collagen, and proteoglycan with interspersed chondrocytes. When undisturbed, chondrocytes show a balance between anabolic and catabolic activity, which maintains the aggrecan structure.[31] A loss of cartilaginous matrix represents an imbalance between the synthesis (anabolic) and resorption (catabolic) of cartilage in the joint. Severe mechanical strain causes an immediate up-regulation of cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) transcription via a "shock-sensor" system in chondrocytes and tendons,[85] while lower levels of mechanical strain, which occur with normal activity or therapeutic exercise, actually inhibit this up-regulation and promote cartilage remodeling through proteoglycan and collagen synthesis. Up-regulation of cytokines leads to induction of matrix metalloproteinases (MMP), which enzymatically degrade the cartilage structure.[30] Mechanical strain also causes microcellular damage leading to release of extracellular membrane and intracellular, microtubule elements into the joint.[44] These mechanical forces also produce other metabolic changes, such as conversion of phospholipids from damaged cell membranes within joints by phospholipase A_2 (PLA_2) to arachidonic acid.[1,4,47,54] Persistent stress on joint tissues along with these metabolic processes leads to inflammation. However, because cartilage is not vascularized or innervated, joint swelling or pain may not occur. Indeed, arthroscopic studies have detected clinically unrecognized synovitis in up to one third of patients with radiographic changes of osteoarthritis with the sites of synovial inflammation being found adjacent to the cartilage lesions.[5]

The superficial zone of articular cartilage obtained from a patient with osteoarthritis and rheumatoid arthritis shows positive immunostaining for IL-1β, TNFα, and inducible nitric oxide synthase.[62] The major cytokines that generate degradative processes in the synovium include

IL-1β, IL-6, interleukin-17 (IL-17), and TNF-α.[55] Other substances, or by-products, which are likely involved in osteoarthritis and further increase cartilage degradation include IGF-1, TGFβ1, and chondrodegradative enzymes.[7] Metabolically based, inflammatory imbalances in synthesis and resorption of cartilage as well as changes in the production of fatty acid metabolites sustain osteoarthritis over time, leading to further cartilage degradation.

METABOLIC ASPECTS OF OSTEOARTHRITIS

Arachidonic acid is the primary fatty acid generated by the metabolic conversion of cell membrane phospholipids by PLA_2 in situations of acute or chronic joint damage. Further enzymatic activity converts arachidonic acid to a variety of inflammatory mediators, which promote further disease progression. Although there can be many causes of the initial inflammatory response, the final common mediators of osteoarthritis are always chemical and a result of metabolic processes, or an overabundance of metabolic substrates, products, or both.[73] These mediators include cytokines and eicosanoids, whose production is greatly impacted by levels of fatty acids in the body and the metabolic processes described above.[3]

Fatty acid levels in bone are 50%-90% higher in patients with osteoarthritis compared to controls.[69] In addition, depending on the severity of osteoarthritis, there is an associated accumulation of total fatty acids and essential fatty acids in the chondrocytes of the joint in patients with osteoarthritis, suggesting a strong involvement of fatty acid metabolism in the etiology and/or pathogenesis of the disease.[50] Clinical studies show strong linkage between metabolic defects in essential fatty acid metabolism and an overabundance of fatty acids that are associated with osteoarthritis.[35]

Arachidonic acid is an essential fatty acid obtained from the diet as well as from the enzymatic conversion of phospholipids from damaged cell membranes. Arachidonic acid is a necessary substrate for membrane building, platelet function, induction of inflammation for tissue repair, and many other functions in the body.[10] With regard to inflammation, the two most important enzymatic pathways for metabolic conversion of arachidonic acid to inflammatory metabolites in the body are mediated by the enzymes 5-lipoxygenase (5-LOX) and cyclooxygenase (COX).[56] These parallel pathways generate leukotrienes and prostaglandins, thromboxanes, and prostacyclins, respectively, which play important roles in the initiation and progression of the inflammatory response. The increased levels of these fatty acid metabolites lead to further damage of cell membranes producing even more arachidonic acid and inflammation in a cyclical process (Figure 1).

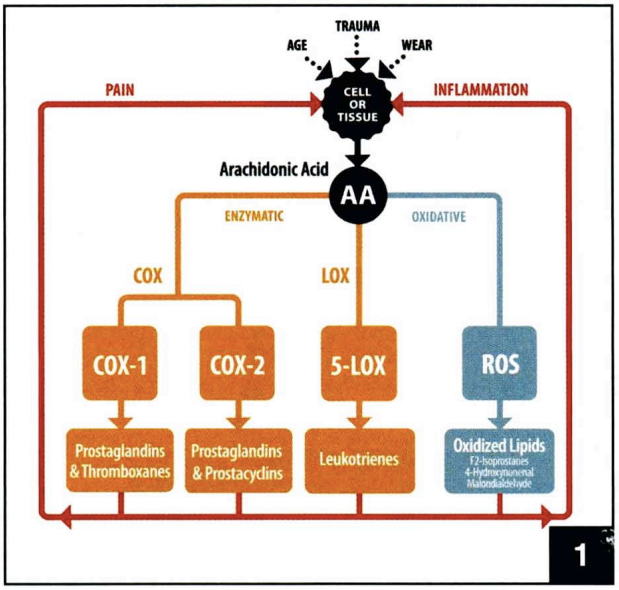

Figure 1. Enzymatic and oxidative metabolism of arachidonic acid (AA) by cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), and reactive oxygen species (ROS) involved in osteoarthritis.

The metabolic conversion of arachidonic acid by COX-1, a constitutively produced enzyme, leads to the generation of prostaglandins and thromboxanes. These fatty acid metabolites help regulate normal physiological functions, such as platelet aggregation, protection of cell function and integrity in the stomach, and maintenance of normal kidney function.[14] COX-2 is constitutively expressed in the kidneys and brain but is inducible by proinflammatory cytokines released by chondrocytes within the joint. This enzyme is required to mount a bodily defense for repair of damaged tissue or to promote immune activation when the body is under a viral assault.[40] Conversion of arachidonic acid by COX-2 leads to the generation of physiologically important prostaglandins for tissue repair and prostacyclins.[90]

The metabolic conversion of arachidonic acid by 5-LOX, another damage-inducible enzyme, leads to the production of leukotrienes. These inflammatory mediators, particularly leukotriene B_4, are chemoattractant, fatty acid metabolites that cause the influx of fluid and cells to the site of tissue damage to aid in the repair of tissue.[88] This, in turn, initiates new rounds of reactive oxygen species production, up-regulation of the cytokine-induced inflammatory cascade, continual breakdown of cellular membranes leading to more arachidonic acid generation, and finally, further chronic expression of COX-2 and 5-LOX over time (Figure 2).[34,76]

Matrix metalloproteinases, produced from chondrocytes, are zinc-containing proteinases that digest cartilage and

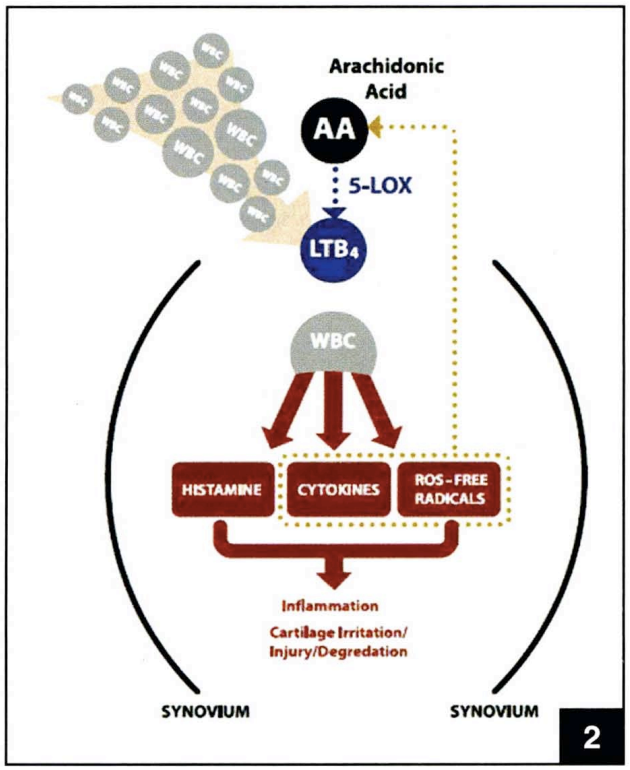

Figure 2. Induction of inflammatory cascade via the 5-lipoxygenase (5-LOX) metabolism of arachidonic acid (AA) to generate leukotriene B_4 (LTB_4), which attracts white blood cells (WBC) to the joint.

cause severe joint damage.[18] The expression of MMP-1 and MMP-13 in particular, induced by IL-1β, causes the digestion of type II collagen, the principal collagenous material in hyaline cartilage.[83] Induction of reactive oxygen species formation by leukotriene B_4 attraction of white blood cells (WBCs) to the joint directly impacts the production of IL-1β and induction of MMP.[88]

Chondrocytes also produce reactive oxygen species.[32,38,61,70] Reactive oxygen species production has been linked to damage to all cartilage matrix components, either by direct or indirect attack, and reduces matrix synthesis. Reactive oxygen species production may also induce apoptosis and/or activate latent metalloproteinases.[39] Another pathway of conversion is the non-enzymatic lipid peroxidation of arachidonic acid. When arachidonic acid is exposed to reactive oxygen species, the molecule is oxidized to three primary products: F2-isoprostanes, 4-hydroxynonenal, and malondialdehyde (Figure 1).[21,71] These chemical arachidonic acid conversion products have been shown to be elevated in synovial fluid, synoviocytes, and serum of patients with osteoarthritis when compared to healthy control subjects.[6] All three molecules are known to directly degrade hyaline cartilage. In addition, other reactive oxygen species found in the joints of patients with

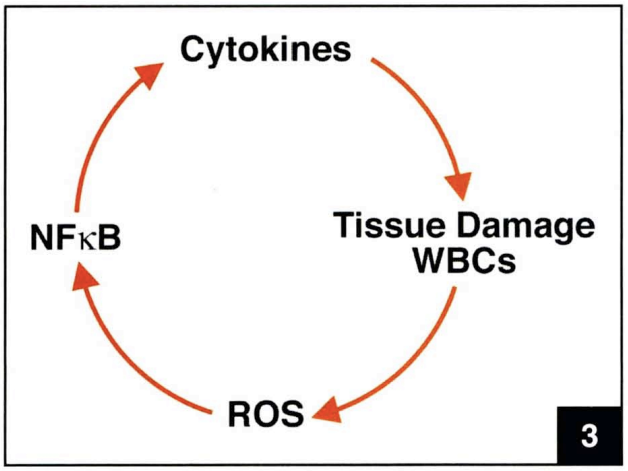

Figure 3. Cyclical induction of nuclear factor kappa B (NFκB) and cytokines via tissue damage-induced white blood cell (WBC) generation of reactive oxygen species (ROS).

osteoarthritis, including superoxide generated by the xanthine-hypoxanthine system, hydroxyl radicals, peroxide, and hydroxyproline, directly degrade cartilage.[39,79,80] Dietary antioxidants have been shown to slow the progression of osteoarthritis.[58,60]

Interleukin-1β induces reactive oxygen species, which promote the expression of the pro-inflammatory transcription factor, nuclear factor kappa B (NFκB).[8] Reactive oxygen species generated by chondrocytes have also been implicated in the pathophysiology of cartilage damage.[25,26,33] Reactive oxygen species-induced NFκB generation then up-regulates expression of COX-2.[40,66,92] Reactive oxygen species are generated by the action of 5-LOX conversion of arachidonic acid to leukotrienes, which attract white blood cells to the joint and induce NADPH oxidase and TNFα.[8] Lymphoid cells stimulated with IL-1β produce reactive oxygen species and activated NFκB whereas inclusion of antioxidants inhibits this (Figure 3).[11]

Cartilage tissue from degenerative joints exhibits accelerated inflammatory metabolism, an effect that can be reproduced in vitro with normal chondrocytes supplemented with exogenous essential fatty acids.[84] Arthritic joints contain elevated levels of lipid and arachidonic acid accumulation with an increasing degree of lesion and histological severity in osteoarthritis.[49,50]

CONCLUSION

Osteoarthritis can be viewed as a disease with multifactorial etiologies with shared metabolic pathogenesis. Irrespective of causation, metabolic mechanisms generate joint inflammation and produce cartilage degradation, which are aggravated by intrinsic and environmental factors associated with advancing age.

ACKNOWLEDGEMENTS

The authors thank Dr Arthur Weaver for his contributions and Richard W. Kang for his extensive help in the preparation of this manuscript.

REFERENCES

1. Abramson SB. Inflammation in osteoarthritis. *J Rheumatol Suppl*. 2004;31:70-76.
2. Anderson JJ, Felson DT. Factors associated with osteoarthritis of the knee in the first national Health and Nutrition Examination Survey (HANES I). Evidence for an association with overweight, race, and physical demands of work. *Am J Epidemiol*. 1988;128:179-189.
3. Aspden RM, Scheven BA, Hutchison JD. Osteoarthritis is a systemic disorder involving stromal cell differentiation and lipid metabolism. *Lancet*. 2001;357:1118-1120.
4. Attur MG, Dave M, Akamatsu M, Katoh M, Amin AR. Osteoarthritis or osteoarthrosis: the definition of inflammation becomes a semantic issue in the genomic era of molecular medicine. *Osteoarthritis Cartilage*. 2002;10:1-4.
5. Ayral X, Dougados M, Listrat V, Bonvarlet JP, Simonnet J, Amor B. Arthroscopic evaluation of chondropathy in osteoarthritis of the knee. *J Rheumatol*. 1996;23:698-706.
6. Basu S, Whiteman M, Mattey DL, Halliwell B. Raised levels of F(2)-isoprostanes and prostaglandin F(2alpha) in different rheumatic diseases. *Ann Rheum Dis*. 2001;60:627-631.
7. Blumenfeld I, Levine E. The role of transforming growth factor (TGF)-beta, insulin-like growth factor (IGF)-1, and interleukin (IL)-1 in osteoarthritis and aging of joints. *Exp Gerontol*. 1999;34:821-829.
8. Bonizzi G, Piette J, Merville MP, Bours V. Cell type-specific role for reactive oxygen species in nuclear factor-kappaB activation by interleukin-1. *Biochem Pharmacol*. 2000;59:7-11.
9. Brandi ML, Gennari L, Cerinic MM, et al. Genetic markers of osteoarticular disorders: facts and hopes. *Arthritis Res*. 2001;3:270-280.
10. Brash AR. Arachidonic acid as a bioactive molecule. *J Clin Invest*. 2001;107:1339-1345.
11. Brigelius-Flohe R, Banning A, Kny M, Bol GF. Redox events in interleukin-1 signaling. *Arch Biochem Biophys*. 2004;423:66-73.
12. Buckwalter JA, Saltzman C, Brown T. The impact of osteoarthritis: implications for research. *Clin Orthop*. 2004;427(Suppl):S6-S15.
13. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol*. 2004;25:4-7.
14. Dannhardt G, Kiefer W. Cyclooxygenase inhibitors—current status and future prospects. *Eur J Med Chem*. 2001;36:109-126.
15. Deschner J, Hofman CR, Piesco NP, Argarwal S. Signal transduction by mechanical strain in chondrocytes. *Curr Opin Clin Nutr Metab Care*. 2003;6:289-293.
16. Dieppe PA, Cushnaghan J, Shepstone L. The Bristol 'OA500' study: progression of osteoarthritis (OA) over 3 years and the relationship between clinical and radiographic changes at the knee joint. *Osteoarthritis Cartilage*. 1997;5:87-97.
17. Dijkgraaf LC, de Bont LG, Boering G, Liem RS. The structure, biochemistry, and metabolism of osteoarthritic cartilage: a review of the literature. *J Oral Maxillofac Surg*. 1995;53:1182-1192.
18. Docherty AJ, Murphy G. The tissue metalloproteinase family and the inhibitor TIMP: a study using cDNAs and recombinant proteins. *Ann Rheum Dis*. 1990;49:469-479.
19. Dougados M, Gueguen A, Nguyen M, et al. Longitudinal radiologic evaluation of osteoarthritis of the knee. *J Rheumatol*. 1992;19:378-384.
20. Dougados M, Gueguen A, Nguyen M, et al. Radiological progression of hip osteoarthritis: definition, risk factors and correlations with clinical status. *Ann Rheum Dis*. 1996;55:356-362.
21. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde, and related aldehydes. *Free Radic Biol Med*. 1991;11:81-128.
22. Favus MJ, Utset TO, Lee C. Nutritional support and management of musculoskeletal diseases. In: Bronner F, ed. *Nutritional Aspects of Clinical Management of Chronic Disorders and Diseases*. Boca Raton, Fla: CRC Press; 2003:129-154.
23. Felson DT. Risk factors for osteoarthritis: understanding joint vulnerability. *Clin Orthop*. 2004;427(Suppl):S16-S21.
24. Felson DT. Weight and osteoarthritis. *J Rheumatol*. 1995;43:7-9.
25. Felson DT, Lawrence RC, Dieppe PA, et al. Osteoarthritis: new insights, part 1: the disease and its risk factors. *Ann Intern Med*. 2000;133:635-646.
26. Felson DT, Lawrence RC, Hochberg MC, et al. Osteoarthritis: new insights, part 2: treatment approaches. *Ann Intern Med*. 2000;133:726-737.
27. Felson DT, Zhang Y. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. *Arthritis Rheum*. 1998;41:1343-1355.
28. Felson DT, Zhang Y, Anthony JM, Naimark A, Anderson JJ. Weight loss reduces the risk for symptomatic knee osteoarthritis in women. The Framingham Study. *Ann Intern Med*. 1992;116:535-539.
29. Felson DT, Zhang Y, Hannan MT, et al. Risk factors for incident radiographic knee osteoarthritis in the elderly: the Framingham Study. *Arthritis Rheum*. 1997;40:728-733.
30. Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. *Biorheology*. 2002;39:237-246.
31. Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clin Orthop*. 2004;427(Suppl):S27-S36.
32. Greenwald RA, Moy WW. Inhibition of collagen gelation by action of the superoxide radical. *Arthritis Rheum*. 1979;22:251-259.
33. Haklar U, Yuksel M, Velioglu A, Turkmen M, Haklar G, Yalcin AS. Oxygen radicals and nitric oxide levels in chondral or meniscal lesions or both. *Clin Orthop*. 2002;403:135-142.
34. Hardy MM, Seibert K, Manning PT, et al. Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. *Arthritis Rheum*. 2002;46:1789-1803.
35. Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J Rheumatol*. 1995;22:1118-1123.
36. Hart DJ, Doyle DV, Spector TD. Incidence and risk factors for radiographic knee osteoarthritis in middle-aged women: the Chingford study. *Arthritis Rheum*. 1999;42:17-24.
37. Hart DJ, Spector TD. The relationship of obesity, fat distri-

bution and osteoarthritis in women in the general population: the Clingford Study. *J Rheumatol*. 1993;20:331-335.
38. Henrotin Y, Deby-Dupont G, Deby C, Franchimont P, Emerit I. Active oxygen species, articular inflammation, and cartilage damage. *EXS*. 1992;62:308-322.
39. Henrotin YE, Bruckner P, Pujol JP. The role of reactive oxygen species in homeostasis and degradation of cartilage. *Osteoarthritis Cartilage*. 2003;11:747-755.
40. Herschman HR. Regulation of prostaglandin synthase-1 and prostaglandin synthase-2. *Cancer Metastasis Rev*. 1994;134:241-256.
41. Herzog W, Longino D, Clark A. The role of muscles in joint adaptation and degeneration. *Langenbecks Arch Surg*. 2003;388:305-315.
42. Horton WE Jr, Lethbridge-Cejku M, Hochberg MC, et al. An association between an aggrecan polymorphic allele and bilateral hand osteoarthritis in elderly white men: data from the Baltimore Longitudinal Study of Aging (BLSA). *Osteoarthritis Cartilage*. 1998;6:245-251.
43. Hunter DJ, Hart D, Snieder H, Bettica P, Swaminathan R, Spector TD. Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins. *Rheumatology (Oxford)*. 2003;42:1311-1316.
44. Jessop JJ, Hoffman T. Production and release of IL-1 beta by human peripheral blood monocytes in response to diverse stimuli: possible role of "microdamage" to account for unregulated release. *Lymphokine Cytokine Res*. 1993;12:51-58.
45. Jimenez SA, Williams CJ, Karasick D. Hereditary osteoarthritis. In: Brandt KD, Doherty M, Lohmander LS, eds. *Osteoarthritis*. Oxford, United Kingdom: Oxford University Press; 1988:31-49.
46. Keen RW, Hart DJ, Lanchbury JS, Spector TD. Association of early osteoarthritis of the knee with a Taq I polymorphism of the vitamin D receptor gene. *Arthritis Rheum*. 1997;40:1444-1449.
47. Lajeunesse D, Reboul P. Subchondral bone in osteoarthritis: a biologic link with articular cartilage leading to abnormal remodeling. *Curr Opin Rheumatol*. 2003;15:628-633.
48. Leveille SG. Musculoskeletal aging. *Curr Opin Rheumatol*. 2004;16:114-118.
49. Lippiello L. Lipid and cell metabolic changes associated with essential fatty acid enrichment of articular chondrocytes. *Proc Soc Exp Biol Med*. 1990;195:282-287.
50. Lippiello L, Walsh T, Fienhold M. The association of lipid abnormalities with tissue pathology in human osteoarthritic articular cartilage. *Metabolism*. 1991;40:571-576.
51. Loughlin J, Sinsheimer JS, Mustafa Z, et al. Association of the vitamin D receptor gene, the type I collagen gene COL1A1, and the estrogen receptor gene in idiopathic osteoarthritis. *J Rheumatol*. 2000;27:779-784.
52. Lozada CJ, Altman RD. Management of osteoarthritis. In: Koopman WJ, ed. *Arthritis and Related Conditions*. Philadelphia, Pa: Lippincott, Williams & Wilkins; 2001:2246.
53. Maetzel A, Makela M, Hawker G, Bombardier C. Osteoarthritis of the hip and knee and mechanical occupational exposure—a systematic overview of the evidence. *J Rheumatol*. 1997;24:1599-1607.
54. Malemud CJ. Cytokines as therapeutic targets for osteoarthritis. *BioDrugs*. 2004;18:23-35.
55. Martel-Pelletier J, Alaaeddine N, Pelletier JP. Cytokines and their role in the pathophysiology of osteoarthritis. *Front Biosci*. 1999;4:694-703.
56. Martel-Pelletier J, Lajeunesse D, Reboul P, Pelletier JP. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. *Ann Rheum Dis*. 2003;62:501-509.
57. McAlindon T, Felson DT. Nutrition: risk of osteoarthritis. *Ann Rheum Dis*. 1997;56:397-402.
58. McAlindon T, Zhang Y, Hanna M, et al. Are risk factors for patellofemoral and tibiofemoral knee osteoarthritis different? *J Rheumatol*. 1996;23:332-337.
59. McAlindon TE, Felson DT, Zhang Y, et al. 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;125:353-359.
60. McAlindon TE, Jacques P, Zhang Y, et al. Do antioxidant micronutrients protect against the development and progression of knee osteoarthritis? *Arthritis Rheum*. 1996;39:648-656.
61. McCord JM. Free radicals and inflammation: protection of synovial fluid by superoxide dismutase. *Science*. 1974;185:529-531.
62. Melchiorri C, Meliconi R, Frizziero L, et al. Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis. *Arthritis Rheum*. 1998;41:2165-2174.
63. Meulenbelt I, Bijkerk C, Miedema HS, et al. A genetic association study of the IGF-I gene and radiological osteoarthritis in a population-based cohort study (the Rotterdam study). *Ann Rheum Dis*. 1998;57:371-374.
64. Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev*. 2000;52:673-751.
65. Miquel J. Nutrition and ageing. *Public Health Nutr*. 2001;4:1385-1388.
66. Nakao S, Ogata, Y, Shimizu-Sasaki E, Yamazaki M, Furuyama S, Sugiya H. Activation of NFkappaB is necessary for IL-1beta-induced cyclooxygenase-2 (COX-2) expression in human gingival fibroblasts. *Mol Cell Biochem*. 2000;209:113-118.
67. O'Reilly SC, Jones A, Muir KR, Doherty M. Quadriceps weakness in knee osteoarthritis: the effect on pain and disability. *Ann Rheum Dis*. 1998;57:588-594.
68. Peat G, McCarney R, Croft P. Knee pain and osteoarthritis in older adults: a review of community burden and current use of primary health care. *Ann Rheum Dis*. 2001;60:91-97.
69. Plumb MS, Aspden RM. High levels of fat and (n-6) fatty acids in cancellous bone in osteoarthritis. *Lipids in Health and Disease*. 2004;3:12.
70. Rathakrishnan C, Tiku K, Raghavan A, Tiku ML. Release of oxygen radicals by articular chondrocytes: a study of luminol-dependent chemoluminescence and hydrogen peroxide secretion. *J Bone Miner Res*. 1992;7:1139-1148.
71. Roberts LJ, Morrow JD. The generation and actions of isoprostanes. *Biochim Biophys Acta*. 1997;1345:121-135.
72. Roos EM. Joint injury causes knee osteoarthritis in young adults. *Curr Opin Rheumatol*. 2005;17:195-200.
73. Sowers M. Epidemiology of risk factors for osteoarthritis: systemic factors. *Curr Opin Rheumatol*. 2001;13:447-451.
74. Spector TD, Campion GD. Generalised osteoarthritis: a hormonally mediated disease. *Ann Rheum Dis*. 1989;48:523-527.
75. Spector TD, Dacre JE, Harris PA, Huskisson EC. Radiological progression of osteoarthritis: an 11 year follow up study of the knee. *Ann Rheum Dis*. 1992;51:1107-1110.
76. Steiner DR, Gonzalez NC, Wood JG. Leukotriene B(4) promotes reactive oxidant generation and leukocyte adherence during acute hypoxia. *J Appl Physiol*. 2001;91:1160-1167.

77. Stockwell RA. Cartilage failure in osteoarthritis: relevance of normal structure and function. A review. *Clin Anat*. 1991;4:161-191.
78. Teixeira S. Bioflavonoids: proanthocyanidins and quercitin and their potential roles in treating musculoskeletal conditions. *J Orthop Sports Phys Ther*. 2002;32:357-363.
79. Tiku ML, Gupta S, Deshmukh DR. Aggrecan degradation in chondrocytes is mediated by reactive oxygen species and protected by antioxidants. *Free Radic Res*. 1999;30:395-405.
80. Tiku ML, Yan YP, Chen KY. Hydroxyl radical formation in chondrocytes and cartilage as detected by electron paramagnetic resonance spectroscopy using spin trapping reagents. *Free Radic Res*. 1998;29:177-187.
81. Uitterlinden AG, Burger H, Huang Q, et al. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. *J Clin Invest*. 1197;100:259-263.
82. Ushiyama T, Ueyama H, Inoue K, Nishioka J, Ohkubo I, Hukuda S. Estrogen receptor gene polymorphism and generalized osteoarthritis. *J Rheumatol*. 1998;25:134-137.
83. Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res*. 2002;4:157-164.
84. Watkins BA, Xu H, Turek JJ. Linoleate impairs collagen synthesis in primary cultures of avian chondrocytes. *Proc Soc Exp Biol Med*. 1996;212:153-159.
85. Westacott CI, Urban JP, Goldring MB, Elson CJ. The effects of pressure on chondrocyte tumour necrosis factor receptor expression. *Biorheology*. 2002;39:125-132.
86. White-O'Connor B, Sobal J, Muncie HL. Dietary habits, weight history and vitamin supplement use in elderly osteoarthritis patients. *J Am Diet Assoc*. 1989;89:378-382.
87. Williams CJ, Jimenez SA. Heritable diseases of cartilage caused by mutations in collagen genes. *J Rheumatol*. 1995;43(Suppl):28-33.
88. Woo CH, Eom YW, Yoo MH, et al. Tumor necrosis factor-alpha generates reactive oxygen species via a cytosolic phospholipase A2-linked cascade. *J Biol Chem*. 2000;275:32357-32362.
89. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. *Bull World Health Organ*. 2003;81:646-656.
90. Wright JM. The double-edged sword of COX-2 selective NSAIDs. *CMAJ*. 2002;167:1131-1137.
91. Yamada Y, Okuizumi H, Miyauchi A, Takagi Y, Ikeda K, Harada A. Association of transforming growth factor beta 1 genotype with spinal osteophytosis in Japanese women. *Arthritis Rheum*. 2000;43:452-460.
92. Yan X, Wu Xiao C, Sun M, Tsang BK, Gibb W. Nuclear factor kappa B activation and regulation of cyclooxygenase type-2 expression in human amnion mesenchymal cells by interleukin-1beta. *Biol Reprod*. 2002;66:1667-1671.
93. Zhou BF, Stamler J, Dennis B, et al. Nutrient intakes of middle-aged men and women in China, Japan, United Kingdom, and United States in the late 1990s: the INTERMAP study. *J Hum Hypertens*. 2003;17:623-630.