

Anti-catabolic/Healing (surgery, injury, aging and overtraining)
Pain-Relief (chronic and acute)

Indications

Significant antioxidant and anti-catabolic benefits indicate Recovery® may be used to reduce spasm and pain and improve rate and quality of post-trauma recovery.

age-related decline, breathing issues, digestive issues, hoof health, pain (back, hock, leg, neck, stifle, etc.), skin and coat issues, trauma rehabilitation, wound healing

The ability to decrease catabolism of cell structures associated with trauma and degenerative disease is what gives Recovery® a potentially broad-spectrum indication profile. Results observed by clinicians over the last 10 years warrant further research for the treatment of chronic skin, respiratory, gastrointestinal and "autoimmune" issues.

Pathophysiology

Trauma, Catabolism and "Dis-ease"

When oxygen is utilized by the body, destructive "exhaust" called reactive oxygen species (ROS) result. ROS include hydroxyl radicals, superoxides, hypochlorite and hydrogen peroxide, etc. Minimal amounts of ROS, play necessary roles in metabolism; whereas, when ROS production increases and the cell's ability to neutralize ROS decreases, tissues are damaged (aging and "dis-ease"). (1-4)

Increased production of ROS is linked to damage, spasm, inflammation, pain and loss of physiological function throughout the body. (5-7)

ROS react with cells initiating chain reactions that result in tissue damage causing inflammation, spasm, pain and disease. (1, 3)

Antioxidants, such as Coenzyme Q10, alpha lipoic acid and NADH (nicotinamide adenine dinucleotide) and anti-catabolic enzymes, such as glutathione peroxidase, superoxide dismutase and catalase minimize the damage due to ROS. Younger healthy cells produce larger quantities of protective substances. (3, 5)

Aging and disease result in diminished cell production of protective compounds leading to increased damage to cell membranes; inevitably, damage to membranes diminishes cellular ability to repair damaged tissue. (1, 7)

Membrane and extra-cellular matrix damage leads to decreased ideal first-intention healing involving parenchyma. (8-10)

Cell damage leads to:

1. Dehydration and Loss of Cell Function
Decreased long chain glycosaminoglycans (GAG's) with an increase in shorter chain GAG's, leads to tissue dehydration and loss of membrane function (9, 11, 12)
2. Loss of Membrane Receptivity to Growth Factors
Cell membrane desensitization to growth factors (somatomedins, insulin, etc.) necessary for cell repair, maintenance, protection and communication (13-15, 41)
3. Sclerosing of Tissue
Deposition of heavily glycosylated, compact & inflexible collagen V & VI (12, 16-22)
4. Compromised Ability to Heal
Increased granulomatous second intention healing involving stromal elements (i.e. development of scar tissue) resulting in loss of cell/tissue function (9, 42)

Consequences:

Loss of cell and tissue function results in further inability to repair damage, leading to increased tendency to bruising, excessive inflammation, spasm, joint stiffness, digestive abnormalities and respiratory distress. (7, 9, 15, 20, 21, 23, 24)

* Insulin shuttles amino acids, glucose, fatty acids, glucosamine and other precursors into the cell so that the cell may synthesize required structures for tissue repair.

Mechanism of Action

Recovery® is designed to halt damage & pain at the "root". (43, 44)

Nutricol®, a potent proprietary bioflavonoid complex containing EGCG, proanthocyanidins, theaflavin and resveratrol from grapes and tea, is the primary active ingredient in Recovery®.

Nutricol® reinforces membrane and matrix structure (helps to halt damage that initiates inflammatory and spasmodic reactions) (26, 27, 31, 45, 46)

Nutricol® increases membrane receptivity to hormones such as insulin, IGF and thyroxine (required for anabolic repair/healing) (13, 14)

Site of Action

Nutricol® embeds in the cell membrane and matrix. (43, 44, 48)

Mechanism of Action

The significant water and fat soluble antioxidant actions of Nutricol® produce the following anti-catabolic and inflammation-modulating effects:

1. Stabilize collagen aldimine reducible cross-links to reinforce the strength and elasticity of connective tissues such as cartilage, synovium, ligaments, tendons, fascia, bone, blood vessel walls and the dermis of the skin.
2. Neutralize ROS and catabolic enzymes decreasing their negative impact on cellular and extra-cellular structure and function; this improves membrane receptivity to growth factors such as insulin, somatomedins and thyroxine required for anabolic repair and cell maintenance (4, 10, 13, 28-30, 35, 49)
3. Decrease excess production of catabolic substances such as collagenase, elastase, hyaluronidase, TNF, NOS and xanthine oxidase*; these substances are released from immune, microbial and damaged cells and cause damage to connective and epithelial tissue, resulting in joint pain, inflammation, capillary fragility and other soft-tissue damage (4, 25, 31-35)
4. Prevent the release of inflammation promoters such as histamine, serine proteases, prostaglandins and leukotrienes by non-competitively inhibiting the release of the pro-inflammatory enzymes cyclo-oxygenase, lipoxygenase and phosphodiesterase (33, 36)
5. Improve protective epithelial mucosal surface integrity (digestive, respiratory & genitourinary tract) (37-40)

*Xanthine oxidase - enzyme that produces ROS. (4, 50)

Ingredients Recovery® EQ-Extra Strength

Nutricol®	1,000mg
methyl sulfonyl methane	10,000mg
glucosamine hydrochloride (vegan-source)	10,000mg
trimethylglycine (TMG)	1,000mg
vitamin C	1,400mg
magnesium (elemental)	525mg
vitamin E (natural d-alpha tocopheryl)	750iu
hyaluronic acid (vegan-source)	100mg

*serving = 1 scoop/26 grams/1 oz/2 tablespoons

Dosage and Administration

Suggested use:

Introduce gradually over a two week period to a concentrated dose of ½ scoop per 300 lbs body weight. Mix with food. After 30 - 60 days it may be possible to reduce intake to ¼ scoop per 300lbs body weight.

*Increasing the dose too rapidly may result in temporary loose stool, fatigue and hypersensitivity at previous trauma sites.

Summary

By implementing Recovery[®] (a Biostructural[®] Medicine), health care professionals can safely modulate inflammatory conditions, prevent tissue damage and improve the quality and rate of healing.

Recovery[®] is believed to decrease trauma (from disease, surgery and injury) by increasing membrane receptivity to growth factors and stabilizing cell structures.

We believe Recovery[®] should be used to halt damage and pain and promote healing.

Safely Combining with Drugs

Due to its antioxidant, inflammation-modulating and anti-catabolic action, combining Recovery[®] with drugs can lead to reduced drug toxicity and side effects:

Anti-inflammatory (NSAIDs/cox-2 inhibitors)

Most conventional NSAIDs interfere with cyclo-oxygenase and prostaglandins. Cell damage still continues because:

1. Oxidation of membranes remains unblocked
2. With standard NSAIDs, the production of PG1 and PG3, normally involved in repair, are also blocked

Recovery[®] benefits alone or combined with NSAIDs include:

1. Inhibiting the inflammatory cascade or "domino effect" by increasing a cell's ability to neutralize lysosomal enzymes and ROS released from neighboring damaged cells - reducing trauma.
2. Increasing delivery of certain hormones, neurochemicals and nutrients into the cell and enhancing waste transport out of the cell - improving cell communication.

Studies demonstrate that the addition of Recovery[®] ingredients with Sulindac (NSAID) results in a reduction in GI side-effects that accompany Sulindac usage (Ohishi et al. Cancer Lett 2002, 177(1):49-56)

Corticosteroids

Corticosteroids mimic cortisol, which reduces inflammation; however, corticosteroids inhibit immune response and ability to repair, predisposing individuals to risk of infection and accelerated rate of tissue breakdown.

Excessive levels of nitric oxide synthase (NOS), an enzyme that produces nitric oxide, are involved in the initiation and progression of cancer and inflammation. Studies have shown higher levels of nitric oxide in various inflammatory bowel diseases, and that corticosteroids have no effect on reducing NOS. (N Leonard, et. al. J. Clin. Pathology: 1998, 51: (10) 750-753)

Recovery[®] may compliment corticosteroids as it can normalize levels of NOS (Yu-Li Lin et.al. Molecular Pharm: 1997 (52):465-472).

Acetaminophen

Recovery[®] ingredients reduce acetaminophen-induced kidney and liver toxicity (Res Commun Mol Pathol Pharmacol 2000; 107(1-2):137-66), (Ray S.D., Arch Biochem Biophys 1999 Sep 1; 369(1):42-58).

Many cases have demonstrated Recovery[®] may be superior to acetaminophen for chronic pain relief. Recovery[®] decreases the need for acetaminophen

Antibiotics

2 studies report anti-bacterial action was enhanced when Recovery[®] ingredients were combined with ampicillin/sulbactam, benzylpenicillin, oxacillin, methacillin, cephalixin (Journal of Antimicrobial Chemotherapy, 2001, (48), 361-364), (Antimicrobial Agents and Chemotherapy, 2001, 45, (6), 1737-1742).

Anti-coagulants

Since 1998, there have been observations with several patients on warfarin and Recovery[®]. There were no changes in prothrombin time reported, nor any signs of increased bleeding. Recovery[®] may have anti-platelet activity related to normalizing excessive platelet adhesiveness. (Kang WS., Thromb Res 1999 Nov 1; 96(3):229-37)

Amiodarone, Doxorubicin, Idarubicin, 4-HC

The ingredients in Recovery[®] reduce organ and serum toxicity induced by these drugs (Bagchi D., Drugs Exp Clin Res 2001; 27(1): 3-15), (Res Commun Mol Pathol Pharmacol 2000; 107(1-2): 137-66)

Safety Data

Recovery[®] has significant benefits with very low risk. All Recovery[®] ingredients are naturally-occurring and non-toxic.

Nutricol[®] constituents have been clinically observed to possess health-promoting properties in the liver, lung, breast, pancreas, bladder, prostate, skin and most of the gastrointestinal system (Fujiki. (1999) J. Cancer Res Clin Oncol.125:589-97).

Effects on Liver Function

Due to anti-catabolic and anti-oxidative actions, Recovery[®] may aid in the proper elimination and metabolism of drugs and other toxins by supporting 4 Phase II liver pathways (glutathione conjugation, taurine conjugation, methylation, and sulfation).

Drug and Food Interactions

Mixing Nutricol[®] with dairy inhibits absorption.

Side effects and precautions

Recovery[®] contains hypoallergenic ingredients; however, the introduction of any new food or drug may result in an allergy.

Toxicology Data

Nutricol[®]

EGCG (epigallocatechin gallate)

The LD50 in male rats is greater than 5g/kg and 3g/kg in female rats. The rats were Sprague-Dawley rats (Yamane et al. (1995) Cancer 7:1662-7). Found to be non-toxic for Rodents and Humans (Fujiki et al. 1998).

Procyanidolic oligomers, resveratrol

The LD50* found to be greater than 5g/kg body weight in a single oral intubation to fasted male and female albino rats. (Bagchi et al. (2000) Toxicology 148:87-197)

Glucosamine (2-amino-2-deoxy-alpha-D-glucose)

No mortalities in mice or rats at very high levels. LD50 is greater than 5g/kg of body weight orally. (Pharmatherapeutica 1982; 3(3):157-68) Theoretically, long-term use of very high-doses of glucosamine may result in hyperglycemia.

*Recovery[®] has demonstrated blood sugar regulating effects. Nutricol[®] increases membrane insulin sensitivity. Recovery[®] is safe to administer to stable Type II diabetics.

MSM (methyl sulfonyl methane)

MSM has very low toxicity, with an LD50 in rats that exceeds 20g/kg body weight per day. In dogs, no toxicity was reported in a 30-day test receiving 3g/kg body weight per day, administered both orally and intravenously. There was a drop in hematocrit in the later stages of the high dose intravenous study that returned to normal post-treatment. (Metcalf, J.W. (1986) MSM status report, Eq. Vet. Data 7:332-334).

TMG (trimethylglycine)

Safety studies show TMG to be very safe, with an acute LD50 in rats of over 11,000 mg/kg body weight. (Life Science Research 1990).

Disclaimer

Administering natural products should be a decision based on personal research and understanding of the role food-derived components play in health and wellbeing.

The information contained within this document is for informational purposes only and is not intended as a substitute for advice from a veterinarian or other health care professional, and should not be used for diagnosis or treatment of any health problem or for prescription of any medication or other treatment. A health care professional should be consulted before starting any diet, exercise or supplementation program, before administering any medication, or if you suspect a health problem. Do not discontinue any other medical treatments without first consulting a veterinarian.

Clinical References

1. Stohs SJ, J Basic Clin Physiol Pharmacol 1995; 6(3-4):205-28 The role of free radicals in toxicity and disease. Oxidative stress associated with production of reactive oxygen species is believed to be involved not only in the toxicity of xenobiotics but also in the pathophysiology of aging, and various age-related diseases, including cataracts, atherosclerosis, neoplastic diseases, diabetes, diabetic retinopathy, chronic inflammatory diseases of the gastrointestinal tract, aging of skin, diseases associated with cartilage, Alzheimer's disease, and other neurological disorders.
2. Jaeschke H, et al, Toxicol Sci 2002 Feb; 65(2):166-176 Because of its unique metabolism and relationship to the gastrointestinal tract, the liver is an important target of the toxicity of drugs, xenobiotics, and oxidative stress.
3. Sen CK., Sports Med 2001; 31(13):891-908 Studies during the past 2 decades suggest that during strenuous exercise, generation of reactive oxygen species (ROS) is elevated to a level that overwhelms tissue antioxidant defense systems. The result is oxidative stress. Although excessive oxidants may cause damage to tissues, lower levels of oxidants in biological cells may act as messenger molecules enabling the function of numerous physiological processes.
4. Lin JK, Chen PC, Ho CT, Lin-Shiau SY., J Agric Food Chem 2000 Jul;48(7):2736-43. Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by theaflavin-3,3'-digallate, (-)-epigallocatechin-3-gallate, and propyl gallate. The antioxidative activity of polyphenols and PG is due not only to their ability to scavenge superoxides but also to their ability to block XO and related oxidative signal transducers.
5. Droge W. free radicals in the physiological control of cell function. Physiol Rev 2002 Jan; 82(1):47-95 Division of Immunochem., Deutsches Krebsforschungszentrum, Heidelberg.
6. Vaziri ND, et al, Hypertension 2002 Jan; 39(1):135-41 Enhanced nitric oxide inactivation and protein nitration by reactive oxygen species in renal insufficiency. Reactive oxygen species (ROS) avidly reacts with nitric oxide (NO) producing cytotoxic reactive nitrogen species capable of nitrating proteins and damaging other molecules.
7. Gillery P, Monboisse JC, Maquart FX, Borel JP, Med Hypotheses 1989 May; 29(1):47-50. Does oxygen free radical increased formation explain long term complications of diabetes mellitus? Oxygen free radicals (OFR) can form by reaction of glyated proteins with molecular oxygen. The most significant complications of diabetes, for example polyneuritis, retinopathy, microangiopathy, perforating ulcers, impaired healing, may depend on the excessive production of OFR by glyated proteins.
8. Lalazar A, et al, Gene 1997 Aug 22; 195(2):235-43 Activation of mesenchymal cells is a central event in the wound healing response of most tissues.
9. Hildebrand KA, Frank CB, Can J Surg 1998 Dec; 41(6):425-9 Scar formation and ligament healing. Injuries to ligaments induce a healing response that is characterized by the formation of a scar. The scar tissue is weaker, larger and creeps more than normal ligament and is associated with an increased amount of minor collagens (types III, V and VI), decreased collagen cross-links and an increased amount of glycosaminoglycans.
10. Monboisse JC, Borel JP, EXS 1992; 62:323-7. Oxidative damage to collagen. Extracellular matrix molecules, such as collagens, are good targets for oxygen free radicals. Collagen is the only protein susceptible to fragmentation by superoxide anion as demonstrated by the liberation of small 4-hydroxyproline-containing-peptides.
11. Roughley P J, Mort J S. Aging and the aggregating proteoglycans of human articular cartilage. Clinical Science 1986; 71: 337-44. With increasing age, there is an overall decrease in long-chain glycosaminoglycan production and an increase in shorter chain glycosaminoglycan production.
12. Nerlich AG, et al, Virchows Arch 1998 Jan; 432(1):67-76 Immunolocalization of major interstitial collagen types in human lumbar intervertebral discs of various ages. Collagens III and VI were significantly increased in areas of minor to advanced degeneration in all anatomical settings, while collagen V showed only minor changes in its staining pattern. In general, histological signs of tissue degeneration coincided with significant quantitative, but also with certain qualitative, changes in the composition of the collagenous disc matrix.
13. Rizvi SI, J Physiol Pharmacol 2001 Sep; 52(3):483-8 Intracellular reduced glutathione content in normal and type 2 diabetic erythrocytes: effect of insulin and (-) epicatechin. A higher content of dietary flavonoids may thus protect diabetic patients against long-term complications.
14. Rizvi SI, Clin Exp Pharmacol Physiol 2001 Sep; 28(9):776-8 Insulin-like effect of (-) epicatechin on erythrocyte membrane acetylcholinesterase activity in type 2 diabetes mellitus.
15. Bradley JL, et al, Acta Neuropathol (Berl) 2000 May; 99(5):539-46 The extracellular matrix of peripheral nerve in diabetic polyneuropathy.
16. Barnes M J. Collagens in atherosclerosis. Collagen and related research 1985; 5: 65-97. Type V and VI collagens are increased in atherosclerotic plaques.
17. Hibbs M S, Hoidal J R, Kang A H. Expression of a metalloproteinase that degrades native type V collagen and denatured collagens by cultured alveolar macrophages. Journal of Clinical Investigation 1987; 80: 1644-50 Glycosylation increases with age and leads to increased stable crosslinking.
18. Mohan P S, Carter W G, Spiro R G. Occurrence of type VI collagen in extracellular matrix of renal glomerulus and its increase in diabetes. Diabetes 1990; 39: 31-7. Type VI collagen is seen most prominently in pathological situations.
19. Narayanan A S, Page R C. Synthesis of type V collagen by fibroblasts derived from normal, inflamed and hyperplastic human connective tissues. Collagen and Related Research 1985; 5: 297-304 An increased content of type V collagen is apparent in inflammatory and proliferative disease, and hypertrophic scars.
20. Hillmann G, et al, Clin Oral Investig 2001 Dec; 5(4):227-35 Immunohistological and morphometric analysis of inflammatory cells in rapidly progressive periodontitis and adult periodontitis. At baseline, the inflamed gingival tissue consists mainly of collagen types V and VI in areas with infiltrates of inflammatory cells.
21. Primorac D, et al, Croat Med J 2001 Aug; 42(4):393-415 Osteogenesis imperfecta (OI), or brittle bone disease, is a heritable disorder characterized by increased bone fragility. In most cases, there is a reduction in the production of normal type I collagen or the synthesis of abnormal collagen as a result of mutations in the type I collagen genes.
22. Kitamura M, et al, Clin Cardiol 2001 Apr; 24(4):325-9 Collagen remodeling and cardiac dysfunction in patients with hypertrophic cardiomyopathy: the significance of type III and VI collagens.
23. CORA TABAK, et al, Am. J. Respir. Crit. Care Med., Volume 164, Number 1, July 2001, 61-64 Chronic Obstructive Pulmonary Disease and Intake of Catechins, Flavonols, and Flavones The MORGEN Study
24. Karran EH, et al, Ann Rheum Dis 1995 Aug; 54(8):662-9 An in vivo model of cartilage degradation that permits the measurement of proteoglycan and collagen in both non-calcified articular cartilage and calcified cartilage compartments.
25. Paquay JB, et al, J Agric Food Chem 2000 Nov; 48(11):5768-72 It is found that catechins are able to protect against nitric oxide (NO^(*)) toxicity in several ways.
26. Rao CN, Rao VH, Steinmann B., Scand J Rheumatol 1983; 12(1):39-42. Bioflavonoid-mediated stabilization of collagen in adjuvant-induced arthritis. In rats with adjuvant-induced arthritis, the effect of (+)-catechin (CA) on the cross linking of collagen was studied. All results may collectively indicate that catechins promote the cross linking of collagen in arthritic animals.
27. Rao CN, Rao VH, Steinmann B., Ital J Biochem 1981 Jul-Aug; 30(4):259-70. Influence of bioflavonoids on the metabolism and cross linking of collagen. The results of the present study indicate that the synthesis of collagen is unaffected, the cross linking of collagen is promoted and the degradation of soluble collagen is decreased in the bioflavonoids treated groups.
28. Matteucci E, Cell Biol Int 2001; 25(8):771-6 Studies show erythrocyte sodium/hydrogen exchange inhibition by (-) epicatechin could be one of the major mechanisms underlying the antiproliferative effects of catechins.
29. Aucamp J, et al, Anticancer Res 1997 Nov-Dec; 17(6D):4381-5 Inhibition of xanthine oxidase by catechins. The liver enzyme, xanthine oxidase (XO) produces uric acid and reactive oxygen species (ROS) during the catabolism of purines. Excess of the former can lead to gout and of the latter to increased oxidative stress.
30. Rao CN, Rao VH, Verbruggen L, Orloff S., Scand J Rheumatol 1980;9(4):280-4. Effect of bioflavonoids on lysosomal acid hydrolases and lysosomal stability in adjuvant-induced arthritis. Results demonstrate the fragility of lysosomes in arthritic tissues. Administration of CA or HR to the arthritic animals was found to have a prophylactic action by stabilizing liver lysosomes and reducing the free lysosomal enzyme activities in serum, liver, kidney and spleen. CA was more effective than HR
31. Yu-Li Lin, MOLECULAR PHARMACOLOGY 52:465-472 (1997). Epigallocatechin-3-gallate Blocks the Induction of Nitric Oxide Synthase by Down-Regulating Lipopolysaccharide-Induced Activity of Transcription Factor Nuclear Factor- B. Nitric oxide (NO) plays an important role in inflammation.
32. L Liu, Abnormalgenesis, Vol 12, 1203-1208, 1991. Catechin could inhibit the metabolism and DNA damage induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a

- tobacco-specific toxin. Results demonstrate that (+)-catechin inhibits the formation of DNA-damaging intermediates by selectively impairing the enzymatic activation of NNK.
33. Chan MM, et al, *Biochem Pharmacol* 1997 Dec 15; 54(12):1281-6 Inhibition of inducible nitric oxide synthase gene expression & enzyme activity by epigallocatechin gallate. Chronic inflammation is implicated as an underlying factor in the pathogenesis of many disorders.
34. Sazuka M, et al, *Biosci Biotechnology Biochem* 1997 Sep; 61(9):1504-6 Inhibition of collagenases from abnormally produced mouse lung cells by catechins. Results suggest that (-)-epigallocatechin gallate inhibit abnormal cell invasion by inhibiting type IV collagenases of the LL2-Lu3 cells.
35. Monboisse JC, Braquet P, Randoux A, Borel JP, *Biochem Pharmacol* 1983 Jan 1; 32(1):53-8. Non-enzymatic degradation of acid-soluble calf skin collagen by superoxide ion: protective effect of flavonoids. This work confirms that collagen may be degraded during the process of inflammation and that some flavonoids are endowed with protective properties.
36. Nakagawa K, et al, *J Agric Food Chem* 1999 Oct; 47(10):3967-73 Catechin supplementation increases antioxidant capacity and prevents phospholipid hydroperoxidation in plasma of humans.
37. Spencer JP, et al, *Antioxid Redox Signal* 2001 Dec; 3(6):1023-39 Bioavailability of flavan-3-ols and procyanidins: gastrointestinal tract influences and their relevance to bioactive forms in vivo. Studies suggest that the major bioactive forms of flavonol monomers and procyanidins in vivo are likely to be metabolites and/or conjugates of epicatechin. One such metabolite, 3'-O-methylepicatechin, has been shown to exert protective effects against oxidative stress-induced cell death.
38. Hara Y., *J Cell Biochem Suppl* 1997; 27:52-8 Influence of tea catechins on the digestive tract. The bactericidal property of catechins plays several roles in the digestive tract. In the small intestine, catechins inhibit alpha-amylase activity, and a certain amount is absorbed into the portal vein. Although catechins are bactericidal, they do not affect lactic acid bacteria.
39. Murakami S, et al, *J Pharm Pharmacol* 1992 Nov; 44(11):926-8 Five catechins, (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin and (-)-epigallocatechin gallate, inhibited gastric H⁺, K⁺ (+)-ATPase activity. These findings suggest that the anti-secretory and anti-ulcerogenic effects of catechins previously reported, are due to their inhibitory activity on gastric H⁺, K⁺ (+)-ATPase.
40. Hassan A, et al, *Methods Find Exp Clin Pharmacol* 1998 Dec; 20(10):849-54 Role of antioxidants in gastric mucosal damage induced by indomethacin in rats. These results suggest that like plasma, the gastric mucosa has an antioxidant capacity and only when this capacity is exhausted are the lesive effects of the oxygen free radicals manifested.
41. Somasundaram R, et al, *J Biol Chem* 2000 Dec 8; 275(49):38170-5 Collagens serve as an extracellular store of bioactive interleukin 2. The binding of certain growth factors and cytokines to components of the extracellular matrix can regulate their local availability and modulate their biological activities.
42. Bensadoun ES, et al, *Eur Respir J* 1997 Dec; 10(12):2731-7 Proteoglycans in granulomatous lung diseases. In this study, we examined the localization of proteoglycans and collagen in the granulomatous lung conditions, sarcoidosis, extrinsic allergic alveolitis (EAA) and tuberculosis (TB).
43. Kajiyama K, *Biosci Biotechnology Biochem* 2001 Dec; 65(12):2638-43 Steric effects on interaction of catechins with lipid bilayers. Trans-type catechins with the galloyl moiety were located on the surface of the lipid bilayer, as well as cis-type catechins with the galloyl moiety, and perturbed the membrane structure.
44. Tsuchiya H., *Chem Biol Interact* 2001 Mar 14; 134(1):41-54 Stereospecificity in membrane effects of catechins. At lower concentrations (5-100 microm), (-)-epigallocatechin gallate and (-)-epicatechin gallate reduced membrane fluidity more significantly than (-)-epicatechin, suggesting that the intensive membrane effect contributes to the potent medicinal utility of (-)-epigallocatechin gallate.
45. Nagasawa T, et al, *Biosci Biotechnol Biochem* 2000 May; 64(5):1004-10 The results of this study show that the antioxidative property of EGCG was effective for suppressing oxidative modification of the skeletal muscle protein induced by electrical stimulation. This finding demonstrates that EGCG has a beneficial effect in vivo on the free radical-mediated oxidative damage to muscle proteins.
46. Erba D., et al, *Journal of Nutrition*. 1999; 129:2130-2134 Observed protective effects can be attributed to epigallocatechin gallate and we cannot exclude contributions by other catechins. These data support a protective effect against oxidative damage.
47. Rao CN, Rao VH, *Ital J Biochem* 1980 Mar-Apr; 29(2):89-101. Effect of bioflavonoids on the urinary excretion of hydroxyproline, hydroxylysyl glycosides and hexosamine in adjuvant arthritis. The effects of (+)-Catechin (AC) and 0--(beta hydroxyethyl) rutosides (HR) on the urinary collagen metabolites were studied up to 49 days in rats with adjuvant-induced arthritis. The elevated levels of urinary total, non-dialysable and dialysable hydroxyproline, hydroxylysyl glycosides and total hexosamine in the arthritic animals were found to be slightly decreased in the acute phase and significantly decreased in the chronic phase of the disease due to the administration of bioflavonoids. Of the two bioflavonoids tests, CA was found to afford more protective action than HR.
48. Nakayama T, et al, *Biofactors* 2000; 13(1-4):147-51 Interaction of catechins with lipid bilayers has been investigated with liposome systems. Epicatechin gallate had the highest affinity for lipid bilayers, followed by epigallocatechin gallate, epicatechin, and epigallocatechin. Epicatechin gallate and epigallocatechin gallate in the surface of lipid bilayer perturbed the membrane structure.
49. Waltner-Law ME, et al, *J Biol Chem* 2002 Sep 20; 277(38):34933-40 Epigallocatechin gallate represses hepatic glucose production. Results demonstrate that changes in the redox state may have beneficial effects for the treatment of diabetes and suggest a potential role for EGCG as an antidiabetic agent.
50. Shi X, et al, *Mol Cell Biochem* 2000 Mar; 206(1-2):125-32 EGCG efficiently scavenges [•]OH radicals with reaction rate of 4.62 x 10¹¹ M⁻¹ sec⁻¹, which is an order of magnitude higher than several well recognized antioxidants, such as ascorbate, glutathione and cysteine. It also scavenges O₂^{•-} radicals as demonstrated by using xanthine and xanthine oxidase system as a source of O₂^{•-} radicals. Through its antioxidant properties, EGCG exhibited a protective effect against DNA damage induced by Cr (VI).

Bioflavonoids & Osteoarthritis

Singh R, Ahmed S, Malemud CJ, Goldberg VM, Haqqi TM, J Orthop Res 2003 Jan;21(1):102-9 **Epigallocatechin-3-gallate selectively inhibits interleukin-1beta-induced activation of mitogen activated protein kinase subgroup c-jun N-terminal kinase in human osteoarthritis chondrocytes.**

Activation of mitogen activated protein kinases (MAPK) is a critical event in pro-inflammatory cytokine-induced signalling cascade in synoviocytes and chondrocytes that lead to the production of several mediators of cartilage damage in an arthritic joint. Green tea (*Camellia sinensis*) is a widely consumed beverage and we earlier showed that polyphenols present in green tea (GTP) inhibit the development of inflammation and cartilage damage in an animal model of arthritis. In this study we evaluated the role of epigallocatechin-3-gallate (EGCG), a green tea polyphenol which mimics its anti-inflammatory effects, in modulating the IL-1beta-induced activation of MAPKs in human chondrocytes. We discovered that EGCG inhibited the IL-1beta-induced phosphorylation of c-Jun N-terminal kinase (JNK) isoforms, accumulation of phospho-c-Jun and DNA binding activity of AP-1 in osteoarthritis (OA) chondrocytes. Also IL-1beta, but not EGCG, induced the expression of JNK p46 without modulating the expression of JNK p54 in OA chondrocytes. In immunocomplex kinase assays, EGCG completely blocked the substrate phosphorylating activity of JNK but not of p38-MAPK. EGCG had no inhibitory effect on the activation of extracellular signal-regulated kinase p44/p42 (ERKp44/p42) or p38-MAPK in OA chondrocytes. EGCG or IL-1beta did not alter the total non-phosphorylated levels of either p38-MAPK or ERKp44/p42 in OA chondrocytes. **Conclusion:** These are novel findings and indicate that EGCG may be of potential benefit in inhibiting IL-1beta-induced catabolic effects in OA chondrocytes that are dependent on JNK activity.

Ahmed S, Rahman A, Hasnain A, Lalonde M, Goldberg VM, Haqqi TM, Free Radic Biol Med 2002 Oct 15;33 (8):1097-105 **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.**

We have previously shown that green tea polyphenols inhibit the onset and severity of collagen II-induced arthritis in mice. In the present study, we report the pharmacological effects of green tea polyphenol epigallocatechin-3-gallate (EGCG), on interleukin-1 beta (IL-1 beta)-induced expression and activity of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in human chondrocytes derived from osteoarthritis (OA) cartilage. Stimulation of human chondrocytes with IL-1 beta (5 ng/ml) for 24 h resulted in significantly enhanced production of nitric oxide (NO) and prostaglandin E(2) (PGE(2)) when compared to untreated controls ($p < .001$). Pretreatment of human chondrocytes with EGCG showed a dose-dependent inhibition in the production of NO and PGE(2) by 48% and 24%, respectively, and correlated with the inhibition of iNOS and COX-2 activities ($p < .005$). In addition, IL-1 beta-induced expression of iNOS and COX-2 was also markedly inhibited in human chondrocytes pretreated with EGCG ($p < .001$). Parallel to these findings, EGCG also inhibited the IL-1 beta-induced LDH release in chondrocytes cultures. **Conclusion:** Overall, the study suggests that EGCG affords protection against IL-1 beta-induced production of catabolic mediators NO and PGE (2) in human chondrocytes by regulating the expression and catalytic activity of their respective enzymes. Furthermore, our results also indicate that EGCG may be of potential therapeutic value for inhibiting cartilage resorption in arthritic joints.

Singh R, Ahmed S, Islam N, Goldberg VM, Haqqi TM, Arthritis Rheum 2002 Aug; 46 (8):2079-86 **Epigallocatechin gallate inhibits interleukin-1beta-induced expression of nitric oxide synthase and production of nitric oxide in human chondrocytes: suppression of nuclear factor kappaB activation by degradation of the inhibitor of nuclear factor kappaB.**

Human chondrocytes were derived from OA cartilage and were treated with EGCG (100 microM) and IL-1beta (2 ng/ml) for different periods, and inducible nitric oxide synthase (iNOS) messenger RNA and protein expression was determined by real-time quantitative reverse transcriptase-polymerase chain reaction and Western blotting, respectively. Production of NO was determined as nitrite in culture supernatant. Activation and translocation of nuclear factor kappaB (NF-kappaB), levels of inhibitor of nuclear factor kappaB (IkpappaB), and NF-kappaB DNA binding activity were determined by Western blotting and a highly sensitive and specific enzyme-linked immunosorbent assay. Activity of IkappaB kinase was determined using in vitro kinase assay. Human chondrocytes cotreated with EGCG produced significantly less NO compared with chondrocytes stimulated with IL-1beta alone ($P < 0.005$). The inhibition of NO production correlated with the suppression of induction and expression of NF-kappaB-dependent gene iNOS. EGCG inhibited the activation and translocation of NF-kappaB to the nucleus by suppressing the degradation of its inhibitory protein IkappaBalpha in the cytoplasm.

Conclusion: Our results indicate that EGCG inhibits the IL-1beta-induced production of NO in human chondrocytes by interfering with the activation of NF-kappaB through a novel mechanism. Our data further suggest that EGCG may be a therapeutically effective inhibitor of IL-1beta-induced inflammatory effects that are dependent on NF-kappaB activation in human OA chondrocytes.

Takita H, Kikuchi M, Sato Y, Kuboki Y, Connect Tissue Res 2002;43(2-3):520-3 **Inhibition of BMP-induced ectopic bone formation by an antiangiogenic agent (epigallocatechin gallate)**

Epigallocatechin gallate (EGCG), which is one of the components of green tea, was recently shown to inhibit endothelial cell growth in vitro and angiogenesis in vivo [5]. We have previously shown that bone and cartilage formation by bone morphogenetic protein (BMP) is highly dependent on the geometry of the carrier (vasculature-inducing or -inhibiting geometry [2]). To verify the function of angiogenesis in the BMP induction system, we examine in this article whether inhibition of angiogenesis enhances chondrogenesis and suppresses osteogenesis. Fibrous glass membrane used as a BMP carrier was mixed with 1.2 micrograms rhBMP-2 and 1-10 micrograms of EGCG and was implanted into rats subcutaneously. As the dose of EGCG increased, alkaline phosphatase activity and calcium content were decreased, whereas the type II collagen content was increased. **Conclusion:** The results clearly indicated that inhibition of vascularization enhanced chondrogenesis and suppressed osteogenesis.

Chen PC, Wheeler DS, Malhotra V, Odoms K, Denenberg AG, Wong HR, Inflammation 2002 Oct; 26 (5):233-41 **A green tea-derived polyphenol, epigallocatechin-3-gallate, inhibits IkappaB kinase activation and IL-8 gene expression in respiratory epithelium.**

Interleukin-8 (IL-8) is a principle neutrophil chemoattractant and activator in humans. There is interest in developing novel pharmacological inhibitors of IL-8 gene expression as a means for modulating inflammation in disease states such as acute lung injury. Herein we determined the effects of epigallocatechin-3-gallate (EGCG), a green tea-derived polyphenol, on tumor necrosis factor-alpha (TNF-alpha)-mediated expression of the IL-8 gene in A549 cells. EGCG inhibited TNF-alpha-mediated IL-8 gene expression in a dose response manner, as measured by ELISA and Northern blot analysis. This effect appears to primarily involve inhibition of IL-8 transcription because EGCG inhibited TNF-alpha-mediated activation of the IL-8 promoter in cells transiently transfected with an IL-8 promoter-luciferase reporter plasmid. In addition, EGCG inhibited TNF-alpha-mediated activation of IkappaB kinase and subsequent activation of the IkappaB alpha/NF-kappaB pathway. **Conclusion:** We conclude that EGCG is a potent inhibitor of IL-8 gene expression in vitro. The proximal mechanism of this effect involves, in part, inhibition of IkappaB kinase activation.

Adcocks C, Collin P, Buttle DJ, J Nutr 2002 Mar; 132 (3):341-6 **Catechins from green tea (*Camellia sinensis*) inhibit bovine and human cartilage proteoglycan and type II collagen degradation in vitro.**

Polyphenolic compounds from green tea have been shown to reduce inflammation in a murine model of inflammatory arthritis, but no studies have been undertaken to investigate whether these compounds are protective to joint tissues. We therefore investigated the effects of catechins found in green tea on cartilage extracellular matrix components using in vitro model systems. Bovine nasal and metacarpophalangeal cartilage as well as human nondiseased, osteoarthritic and rheumatoid cartilage were cultured with and without reagents known to accelerate cartilage matrix breakdown. Individual catechins were added to the cultures and the amount of released proteoglycan and type II collagen was measured by metachromatic assay and inhibition ELISA, respectively. Possible nonspecific or toxic effects of the catechins were assessed by lactate output and proteoglycan synthesis. Catechins, particularly those containing a gallate ester, were effective at micromolar concentrations at inhibiting proteoglycan and type II collagen breakdown. No toxic effects of the catechins were evident. **Conclusion:** We conclude that some green tea catechins are chondroprotective and that consumption of green tea may be prophylactic for arthritis and may benefit the arthritis patient by reducing inflammation and slowing cartilage breakdown. Further studies will be required to determine whether these compounds access the joint space in sufficient concentration and in a form capable of providing efficacy in vivo.