Bioflavonoids: Proanthocyanidins and Quercetin and Their Potential Roles in Treating Musculoskeletal Conditions

Shan Teixeira, PT, MOMT [1]

As a clinician treating musculoskeletal conditions, one is continually in search of safe and more effective treatment methods that will hasten tissue healing. Chronic inflammation has been shown to cause connective tissue degradation. Typically, nonsteroidal anti-inflammatory drugs (NSAIDs) and/or corticosteroids are used to control the inflammatory process, however, long-term use has been associated with potentially serious side effects. The purpose of this article is to introduce and describe literature on 2 natural compounds, namely, proanthocyanidin (PCO) and quercetin, which are 2 specific types of bioflavonoids, and to discuss their potential benefits in treating musculoskeletal conditions. There is evidence to suggest that flavonoids may be beneficial to connective tissue for several reasons, which include the limiting of inflammation and associated tissue degradation, the improvement of local circulation, as well as the promoting of a strong collagen matrix. An overview of bioflavonoids as well as relevant research, safety issues, absorption, and specific sources of PCO and quercetin in foods and through supplementation is included. *J Orthop Sports Phys Ther 2002;32:357–363.*

Key Words: *connective tissue, inflammation, proteolytic enzymes*

Clinicians who treat musculoskeletal injuries and dysfunction often deal with chronic or recalcitrant problems that may not respond readily to traditional methods of treatment. In addition to exercise, manual techniques and various modalities, many clinicians have also considered nutrition and dietary supplements to assist in promoting tissue healing. Despite the increasing interest in nutrition and the use of nutritional supplements, to date, there is a relative paucity of information or research pertaining to nutrition or supplements found in the orthopedic, sports or physical medicine literature.

The purpose of this paper is to introduce and review research pertaining to 2 natural plant compounds, namely, proanthocyanidins (PCO) and quercetin, and to discuss their potential benefits to connective tissue. These benefits may include limiting inflammation and associated tissue degradation, improving local circulation, as well as promoting a stronger collagen matrix.

BACKGROUND

Proanthocyanidins and quercetin are types of bioflavonoids. Bioflavonoids are ubiquitous, naturally occurring compounds present in most plants and are responsible for the bright colors of many fruits and vegetables, as well as the autumn foliage. They were discovered in 1937 by Hungarian biochemist Albert Szent-Gyorgyi, who won a Nobel prize for his research on bioflavonoids and vitamin C. Approximately 4000 flavonoid compounds have been identified. Common groups include procyanidolic oligomers, flavones, flavonols (including quercetin), and isoflavones.

Proanthocyanidins, also known as PCO for procyanidolic oligomers, have been shown to possess many health benefits. Particular attention has been given to their ability to reduce cardiovascular disease. A 1979 *Lancet* publication described the so-called ''French paradox,'' which referred to the fact that despite a higher intake of saturated fat, French people had a lower incidence of cardiovascular disease compared to other countries with similar dietary habits.[45]

This was attributed, in part, to the higher consumption of red wine, which contains the bioflavonoid PCO. This conclusion is further supported in more recent studies by Hertog et al[13,15] that have found an inverse correlation between the amount of flavonoid intake and heart disease.

[1] *Physical therapist, Huber Associates, Auburn, ME.*
Send correspondence to Shan Teixeira, Huber Associates, 637 Minot Avenue, Auburn, ME 04210. E-mail: shan.tex@verizon.net

Inflammation and Tissue Degradation

Inflammation is a normal biological process and the primary means by which tissue healing is initiated and infection is limited in the human body. However, chronic inflammation has been shown to cause connective tissue degradation. This occurs through 2 primary mechanisms: (1) proteolytic enzymes and (2) oxygen-free radicals such as superoxide ion (O^2), singlet, and hydroxyl radicals.[4,8,9]

Proteolytic enzymes including elastase, collagenase, and hyaluronidase are involved in the inflammatory process and are associated with the presence of polymorphonuclear leukocytes (PMNs) and macrophages. They have been shown to be more prevalent in tissue with chronic conditions and to cause degradation of collagen, elastin, and hyaluronic acid.[29]

Superoxide radicals have also been demonstrated to have adverse effects on connective tissue elements. They are produced from polymorphonuclear leukocytes during phagocytosis. Superoxide radicals have been shown to have a direct degradative action on proteoglycans including hyaluronic acid.[33] They also impede the normal gelation of collagen.[9] Superoxide radicals have been implicated as a contributing factor in connective tissue and cartilage degeneration in inflammatory and arthritic conditions. This may occur, in part, due to a change in synovial fluid viscosity.[8]

Nonsteroidal Anti-inflammatory Drugs (NSAIDs) and Corticosteroids

Inflammation and its associated pain are commonly treated with NSAIDs, which are believed to work mainly by inhibiting the enzyme cyclooxygenase.

This enzyme, along with phospholipase A_2, and lipoxygenase, are responsible for converting arachidonic acid into various prostaglandins and thromboxanes that promote the inflammatory process and sensitize peripheral pain receptors.[1]

Corticosteroids are also frequently used to inhibit inflammation. They also inhibit prostaglandin production. Their primary mode of action is believed to be through blocking the enzyme phospholipase A_2.

Many side effects have been reported with the use of both NSAIDs and corticosteroids. The primary side effects associated with NSAID use are related to the gastrointestinal (GI) tract. These include pain, nausea, constipation, diarrhea, hemorrhage, and perforated ulcers. Peptic ulceration has been reported to occur in 5% to 25% of patients using NSAIDs.[44] Renal complications have been reported in 5% of patients receiving NSAID therapy.[49] There is also some evidence to suggest that NSAIDs may interfere with the metabolism of articular cartilage and repair of bone.[42]

The second generation of NSAIDs, including Celebrex (celecoxib) and Vioxx (rofecoxib), which became available in 1999, may reduce the incidence of gastrointestinal complications by selectively inhibiting cyclooxygenase-2 (COX–2), and sparing the cyclooxygenase-1 enzyme. Long-term studies, however, are still not available.

Side effects associated with the use of corticosteroids are similar to NSAIDs in relation to GI symptoms and complications. Long-term use may also cause osteoporosis, avascular necrosis, adrenal insufficiency, predisposition to infection, and the onset of diabetes mellitus.[1] Corticosteroids also decrease the synthesis of collagen and proteoglycan found in connective tissue.

RESEARCH ON FLAVONOIDS

PCOs have been shown to be effective as free radical scavengers and overall antioxidants.[2,4,7,29,35,37,39] These compounds bind to heavy metal ions, which are known to lead to the formation of free radicals. They also inhibit the formation of various oxygen free radicals. Maffei et al[29] reported that PCOs were able to noncompetitively inhibit xanthine oxidase, the promoter of superoxide formation.

In addition, Maffei et al[29] demonstrated that PCOs could significantly inhibit the activity of the proteolytic enzymes collagenase, elastase, hyaluronidase, and β-glucuronidase. These enzymes, as previously mentioned, are directly involved in the turnover of the main structural components of connective tissue including collagen, elastin, and hyaluronic acid.

A study by Tixier et al[46] also found PCOs to have a protective or sparing effect on connective tissue. They found PCOs were able to effectively bind to elastin in the ligamentum nuchae of young rabbits and prevent catabolic degradation by the proteolytic enzyme elastase. The authors indicate that the high affinity of flavonoids for collagen and elastin may be related to the relatively high content of the amino acid proline found within these connective tissues.

Though PCOs have an inhibitory activity upon most enzymes, they have been shown to facilitate the enzyme proline hydroxylase.[3] The hydroxylation of proline is essential to the synthesis of collagen. Masquelier et al[31] reported that PCOs were able to stabilize and increase the cross-linkage of collagen fibrils. This would help promote the strength and overall maturation of the connective tissue.

Quercetin, another bioflavonoid, has been shown to exhibit anti-inflammatory properties through inhibition of various enzymes. Ferandiz et al[7] reported that quercetin was able to modulate arachidonic acid metabolism via inhibition of the enzyme lipoxygenase. Another study by Lee et al[27] indicated that quercetin inhibited phospholipase A_2 of stimu-

lated neutrophils. The authors also reported that quercetin was able to inhibit xanthine oxidase generation of superoxide radicals by 33% and reduce the release of the proteolytic enzyme β-glucuronidase by 52% in vitro. Middleton et al[34] confirmed the inhibition of histamine release by quercetin.

Similar to quercetin, PCOs also may have more direct anti-inflammatory effects. They have been shown to inhibit histamine, serine proteases, prostaglandins and leukotrines.[41]

Safe Flavonoid Intake

Over the past few decades, numerous studies have demonstrated that flavonoids are safe and produce minimal clinical side effects. Masquelier[32] reports that PCOs are nontoxic, even at somewhat high doses (132 mg per lb (.45 kg) body weight per day for 12 months in dogs). Quercetin, similarly, has been reported to be without apparent side effects. Studies conducted on rats and rabbits have found quercetin to be safe even in large quantities (2000 mg per kg of body weight and 5%–10% of total diet) administered for up to 2 years.[16,17,22,23]

Skibola et al[43] indicate that there is ample evidence that a flavonoid-rich diet promotes good health. They do, however, discourage one from carelessly taking excessively high amounts of flavonoids, with the common misconception that if something is good then more is better. They report that flavonoid amounts available through dietary intake alone, as with a typical vegetarian diet, are unlikely to cause adverse health effects. They indicate, however, that at excessive levels (10 to 20 times what can be consumed through dietary means) from large amounts of highly concentrated supplements, flavonoids may act as potential mutagens or as inhibitors of key enzymes.[43] The authors cite Kuhnau,[26] who estimated that the daily dietary intake of mixed flavonoids in the US was in the range of 500 to 1000 mg.[12] At these levels, one would have to consume from 5000 to 20,000 mg of flavonoids to exceed 10 to 20 times the equivalent to the typical dietary intake. These levels are very high and even with supplementation, are quite unrealistic. As a precautionary measure, it is recommended that individuals take 1 g or less per day of flavonoids in the form of supplementation.[11,43]

Lehaun et al[28] did not observe any side effects with the use of 1 g of flavonoids or less per day in adult human patients. Micronized flavonoids in Daflon 500 mg were shown to be tolerated well with no acute or chronic toxicity in patients with chronic venous insufficiency.[18] Flavonoids are well metabolized without significant residuals accumulating in the body, which may contribute to their low toxicity. As with any other compounds, some individuals may be sensitive and develop allergic reactions.

Absorption of Quercetin and PCO

There has been some question as to the bioavailability and absorption of bioflavonoids. It has been stated that flavonoids present in foods cannot be absorbed in the intestine because they are bound to sugars as glycosides. Hollman et al[19] studied dietary absorption of quercetin in healthy subjects with ileostomy who had full, functional small intestines. They reportedly chose subjects with ileostomy to eliminate the effects of quercetin degradation that occurs in the colon, which could lead to an overestimation of the amount absorbed. Absorption of quercetin glycosides from onions was found to be 52%, where 17% of quercetin-3-rutinoside from tea was absorbed. The authors surmised that actual absorption might be higher than these values, for absorbed flavonoids may be re-excreted with bile as was found with rats.[10,47] The authors concluded that humans absorb appreciable amounts of quercetin.[19] Later research by Hollman and Katan[21] further supported the absorption of quercetin.

PCOs have also been reported to be well absorbed. Masquelier[32] notes that PCOs are water soluble and within 60 minutes are well distributed throughout the body. They have a relatively long half-life of 7 hours.

Flavonoids and Foods

The World Health Organization (WHO) study group on diet, nutrition, and prevention of communicable diseases recommends the ingestion of at least 400 g (14 oz) of fruits and vegetables per day.[5] A 1990 survey of American eating habits showed that only 1 in 11 Americans met the guidelines for eating at least 3 servings of vegetables and 2 servings of fruit per day. In fact, 1 in 9 Americans surveyed ate no fruit and no vegetables on the day of the survey, and 45% reported eating no fruit that day.[36]

A partial list of foods, which are generally considered to possess a higher content of these flavonoids, are included in the table. The values listed for quercetin are derived from research done by Hertog et al.[14] They conducted a detailed analysis of 28 vegetables and 9 fruits sampled throughout the year in the Netherlands. The vegetables with the highest reported quercetin content included onions, kale, broccoli, and French beans. Among fruit, apples were found on average to possess the highest amount of quercetin. The herbs parsley and sage were also found to be good sources of quercetin. The values for these spices as well as all the PCO values in the table were taken from Herrmann.[12]

In general, flavonoid levels in processed foods were approximately 50% lower than in fresh products.[14] Though specific flavonoid content in food sources is believed to be somewhat variable, veg-

TABLE. Proanthocyanidin (PCO) and quercetin content of selected foods, in mg per 100-g (3.5-oz) serving.

	Proanthocyanidin (PCO)*	Quercetin†
Vegetables		
Bean (French)	—	4
Bean (broad)	—	2
Broccoli	—	3
Brussels sprout	—	< 0.01
Cabbage (red)	25	0.46
Cauliflower	—	< 0.01
Cucumber	—	< 0.01
Kale	—	11
Lettuce	—	1.4
Mushroom	—	< 0.01
Onions	0–25	35
Radish	—	< 0.01
Rhubarb	200	—
Sauerkraut	—	< 0.01
Spinach	—	< 0.01
Tomato	—	0.8
Turnip tops	—	0.73
Fruits (including peel)		
Apple	20	3.6
Apricot	—	2.5
Blueberry	130–250	—
Cherry (sweet)	6–7	3.2
Currants (black)	130–400	—
Cranberry	60–200	—
Grape (red)	65–140	1.5
Grape (white)	—	1.2
Hawthorn berry	200	—
Pears	—	0.32
Plums (blue)	10–25	0.09
Raspberry (black)	300–400	—
Raspberry (red)	30–35	—
Strawberry	15–35	0.86
Other		
Parsley	—	1400
Sage	—	1000–1500
Wine (red)	100–150	2–4

* Values for PCO from Herrmann.[12]
† Values for quercetin from Hertog et al,[15] except for parsley, sage, and red wine from Herrmann.[12]
Dash (—) indicates values less than 1 mg per 100 g or values unavailable.

etables typically contain more quercetin, whereas fruits, which include berries, provide higher levels of PCO.

Hertog et al[14] commented on the fact that their values were somewhat lower than values reported earlier by Herrmann et al.[12] They attributed their lower quercetin values to the fact that they only analyzed the edible parts of foods where Herrmann et al[12] typically included whole foods. Hertog et al[14] also noted that their more recent study utilized more specific and accurate methods of analysis. Therefore, the values listed for PCO in the table from Herrmann et al[12] may be somewhat higher than actual content. This may also explain why the authors reported such a large range for many selected foods.

Supplementation

PCO and quercetin are also available as supplements. Common sources for PCO include grape seed extract (Vitis vinifera) and pine bark (pycnogenol). Grape seed generally yields the highest concentration of PCO at 95%.[32] Quercetin is available in capsules and is often combined with vitamin C. Blackcurrant, black cherry, and Concord grape liquid concentrates are also available and are excellent sources of flavonoids.

DISCUSSION

Over the past decade, there has been extensive clinical research on flavonoids. To date, the focus of most of the research has been on their antioxidant properties with respect to cardiovascular[2,20,48,50] and peripheral vascular disease.[6,18,29] These studies further validate earlier research suggesting that flavonoids are effective free radical scavengers, and inhibitors of proteolytic enzymes. As stated earlier, oxygen-derived free radicals, and proteolytic enzymes collectively have been associated with connective tissue degradation. In fact, the same enzymes and free radicals implicated in cardiovascular and peripheral vascular disease are also responsible for connective tissue breakdown with musculoskeletal injuries, chronic dysfunction, or disease.[4,8,9,30]

Mantle et al[30] demonstrated that plant flavonoids have the capacity to inhibit a wide range of protease activities in human muscle tissue (rectus abdominis) in vitro. The authors indicate that in addition to their well-described antioxidant characteristics, flavonoids also represent a previously unrecognized source of antiproteolytic compounds, with minimal toxic side effects. They go on to suggest that the therapeutic use of flavonoids may also be of potential benefit in treating muscle-wasting disorders such as muscular dystrophy.

As clinicians treating musculoskeletal conditions, our ultimate goal is not only to eliminate pain and restore function, but also to reduce the incidence and likelihood of subsequent reinjury. Immature collagen fibrils are poorly aligned and, until remodeling has occurred, are more susceptible to permanent deformation under loading. This contributes to the incidence of subsequent musculoskeletal reinjury and chronicity.

In addition to their free radical scavenger and anti-inflammatory effects, there is evidence to suggest that flavonoids, specifically PCOs, have the ability to increase the strength and stability of collagen fibrils.[31,38,40] As novel anti-inflammatory agents, inhibitors of tissue degradation, as well as promoters of a strong connective tissue matrix, flavonoids may be beneficial at all stages of healing and even as a pro-

phylactic measure against musculoskeletal injuries.

Dietary intake of flavonoids has not only been found to be safe, but also positively associated with improved health.[2,11,15,20,24,25,29,48,50] However, flavonoid intake should be encouraged by means of regular consumption of naturally occurring food sources such as fruits and vegetables.

The encouragement of a healthy lifestyle, including smoking cessation, regular exercise, proper body mechanics, and ergonomic principles, should include basic dietary habits in its recommendations. To this end, one should encourage patients to ingest at least 5 servings of fruits and vegetables per day as advised by the National Cancer Institute and the World Health Organization.[5]

In addition to their flavonoid content, fruits and vegetables are naturally low in saturated fat, cholesterol, calories, and sodium, and are rich in potassium, fiber, folic acid, and vitamin C. Patients with restricted or specific dietary needs, such as individuals with diabetes or women during pregnancy, should be cleared by their physician or dietician before making any changes to their diets. Flavonoid supplementation may be considered in patients, who, despite encouragement, will not ingest adequate amounts of fruits and vegetables.

While specific daily requirements of flavonoids have yet to be determined, several studies have demonstrated an inverse relationship between dietary intake of flavonoids and the incidence of stroke[24] and coronary heart disease.[13,15,25]

Despite these benefits, the clinical effects of human exposure to potentially very high levels of flavonoids from concentrated sources remain largely unknown. As a precaution, it is generally advised to keep supplementation levels of flavonoids at 1 g or less per day.

Connective tissue and collagen found in articular cartilage are maintained and repaired through either a direct blood supply or distribution from adjacent tissues. Clinicians commonly see patients with injuries and degenerative conditions involving tissues with inherently more tenuous local circulation. A few examples of this include portions of the rotator cuff, intervertebral discs, and articular cartilage. In addition to the aforementioned benefits of flavonoids on connective tissue elements, they may also have positive indirect effects as well. PCO has been shown to exhibit positive effects on the biochemical properties of blood vessels.[6,29] Maffei et al[29] found PCO to be effective in limiting capillary breakdown in vitro, by preventing oxidant-induced injury and inhibiting enzymes involved in the degradation of the extravascular matrix. Considering the importance of adequate circulation to all types of tissue, including connective tissue and collagen found in articular cartilage, and PCO's proven effects on capillary integrity, one would expect that PCO might also have indirect beneficial effects on musculoskeletal tissue by the preservation of local tissue circulation.

Because NSAIDs and corticosteroids are commonly prescribed in the management of musculoskeletal conditions, their primary mode of action and potential side effects were briefly mentioned. Because much of the research on bioflavonoids has been conducted primarily at the cellular level (in vitro), it is difficult to accurately compare the clinical efficacy of bioflavonoids to that of NSAIDs or corticosteroids.

Despite the increasing clinical use and widespread interest in nutrition and supplementation in the treatment of musculoskeletal conditions, there remains a disproportionately low amount of research conducted and published in the orthopedic, sports, manual therapy, and rehabilitative journals. This may be due, in part, to the perception that so-called ''alternative therapies'' are purely empirical and not based on sound scientific principles. Regardless of whether or not this is a fair representation, this popular belief cannot be effectively challenged without research and statistical data to either support or discourage their continued use.

CONCLUSION

This paper has described how chronic inflammation, by way of proteolytic enzymes and oxygen free radicals, can be damaging to connective tissue. PCO's ability to limit tissue degradation may be due to several mechanisms, including inhibition of proteolytic enzymes, limiting of the production of oxygen free radicals, improving local tissue circulation, and promoting strength and stability of collagen fibrils by facilitating the hydroxylation of proline. PCO also exhibits direct anti-inflammatory effects through inhibition of histamine, prostaglandins and leukotrines. Quercetin was also shown to possess anti-inflammatory properties, and to inhibit free radicals that may lead to tissue degradation.

As novel anti-inflammatory agents, inhibitors of tissue degradation, as well as promoters of a strong connective tissue matrix, flavonoids may be beneficial at all stages of healing. Flavonoids may be particularly useful for chronic or recalcitrant conditions where repeated or long-term use of NSAIDs may not be recommended. Considering their many potential positive effects, coupled with their apparent low toxicity, bioflavonoids including PCO and quercetin may be an effective adjunct to traditional treatment of musculoskeletal conditions. More specific research, including human in vivo studies, are still needed to further establish clinical efficacy and specific guidelines for usage of PCO and quercetin in the management of musculoskeletal conditions.

ACKNOWLEDGEMENTS

The author would like to thank Mark McMahon, PT, for his assistance with editing earlier versions and Michael Marley for his assistance with later versions of this manuscript.

REFERENCES

1. Cain SD, Janos SC. Clinical pharmacology for the physical therapist. In: Boissonnault WG, ed. *Examination in Physical Therapy Practice: Screening for Medical Disease*. New York, NY: Churchill Livingstone; 1995:321–351.
2. Catapano AL. Antioxidant effect of flavonoids. *Angiology.* 1997;48:39–44.
3. Cetta G, Balduini C, Valli M, et al. *Adv Inborn Errors Metab.* 1979;1:143.
4. de Groot H, Rauen U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. *Fundam Clin Pharmacol.* 1998;12:249–255.
5. Diet, nutrition and the prevention of chronic diseases. A report of the WHO Study Group on Diet, Nutrition and Prevention of Noncommunicable Diseases. *Nutr Rev.* 1991;49:291–301.
6. Drubaix I, Robert L, Maraval M, Robert AM. Synthesis of glycoconjugates by human diseased veins: modulation by procyanidolic oligomers. *Int J Exp Pathol.* 1997;78:117–121.
7. Ferrandiz ML, Alcaraz MJ. Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. *Agents Actions.* 1991;32:283–288.
8. Greenwald RA. Effects of oxygen-derived free radicals on connective tissue macromolecules: inhibition by copper-penicillamine complex. *J Rheumatol Suppl.* 1981;7:9–13.
9. Greenwald RA, Moy WW. Inhibition of collagen gelation by action of the superoxide radical. *Arthritis Rheum.* 1979;22:251–259.
10. Harmand MF, Blaquet P. The fate of total flavonolic oligomers extracted from the "Vitis vinifera L" in the rat. *Eur J Drug Metab Pharmacokinet.* 1978;1:15–30.
11. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol.* 1983;32:1141–1148.
12. Herrmann K. Flavonols and flavones in food plants: a review. *J Food Technol.* 1976;11:433–448.
13. Hertog MG, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet.* 1993;342:1007–1011.
14. Hertog MG, Hollman PC, Katan MB. Content of potentially anti-carcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem.* 1992;40:2379-2383.
15. Hertog MG, Kromhout D, Aravanis C, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med.* 1995;155:381–386.
16. Hirono I, Ueno I, Hosaka S, et al. Carcinogenicity examination of quercetin and rutin in ACI rats. *Cancer Lett.* 1981;13:15–21.
17. Hirose M, Fukushima S, Sakata T, Inui M, Ito N. Effect of quercetin on two-stage carcinogenesis of the rat urinary bladder. *Cancer Lett.* 1983;21:23–27.
18. Hitzenberger G. Therapeutic effectiveness of flavonoids illustrated by Daflon 500 mg. *Wien Med Wochenschr.* 1997;147:409–412.
19. Hollman PC, de Vries JH, van Leeuwen SD, Mengelers MJ, Katan MB. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Am J Clin Nutr.* 1995;62:1276–1282.
20. Hollman PC, Hertog MG, Katan MB. Role of dietary flavonoids in protection against cancer and coronary heart disease. *Biochem Soc Trans.* 1996;24:785–789.
21. Hollman PC, Katan MB. Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed Pharmacother.* 1997;51:305–310.
22. Hosaka S, Hirono I. Carcinogenicity test of quercetin by pulmonary-adenoma bioassay in strain a mice. *Gann.* 1981;72:327–328.
23. Kato K, Mori H, Fujii M, et al. Lack of promotive effect of quercetin on methylazoxymethanol acetate carcinogenesis in rats. *J Toxicol Sci.* 1984;9:319–325.
24. Keli SO, Hertog MG, Feskens EJ, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. *Arch Intern Med.* 1996;156:637–642.
25. Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ.* 1996;312:478–481.
26. Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet.* 1976;24:117–191.
27. Lee TP, Matteliano ML, Middleton E Jr. Effects of quercetin on human polymorphonuclear leukocyte lysosomal enzyme release and phospholipid metabolism. *Life Sci.* 1982;31:2765.
28. Lehaun H, Perucker H. *In Methoden der Organischen Chemie.* Stuttgard, Germany: Thieme Verlag; 1975:962.
29. Maffei Facino R, Carini M, Aldini G, Bombardelli E, Morazzoni P, Morelli R. Free radicals scavenging action and anti-enzyme activities of procyanidines from Vitis vinifera. A mechanism for their capillary protective action. *Arzneimittelforschung.* 1994;44:592–601.
30. Mantle D, Falkous G, Perry EK. Effect of flavonoids on protease activities in human skeletal muscle tissue in vitro. *Clin Chim Acta.* 1999;285:13–20.
31. Masquerlier J, Dumon MC, Dumas J. Stabilization of collagen by procyanidolic oligomers. *Acta Ther.* 1981;7:101–105.
32. Masquelier J. Procyanidolic oligomers. *J Parfums Cosmet Arom.* 1990;95:89–97.
33. McCord JM. Free radicals and inflammation: Protection of synovial fluid by superoxide dismutase. *Science.* 1974;185:529–531.
34. Middleton E, Jr., Drzewiecki G, Krishnarao D. Quercetin: An inhibitor of antigen-induced human basophil histamine release. *J Immunol.* 1981;127:546–550.
35. Nagao A, Seki M, Kobayashi H. Inhibition of xanthine oxidase by flavonoids. *Biosci Biotechnol Biochem.* 1999;63:1787–1790.
36. Patterson BH, Block G, Rosenberger WF, Pee D, Kahle LL. Fruit and vegetables in the American diet: data from the NHANES II survey. *Am J Public Health.* 1990;80:1443–1449.
37. Pietta PG. Flavonoids as antioxidants. *J Nat Prod.* 2000;63:1035–1042.
38. Rao CN, Rao VH, Steinmann B. Influence of bioflavonoids on the metabolism and crosslinking of collagen. *Ital J Biochem.* 1981;30:259–270.
39. Robak J, Gryglewski RJ. Bioactivity of flavonoids. *Pol J Pharmacol.* 1996;48:555–564.

40. Ronziere MC, Herbage D, Garrone R, Frey J. Influence of some flavonoids on reticulation of collagen fibrils in vitro. *Biochem Pharmacol.* 1981;30:1771–1776.
41. Schwitters B, Masquelier J. *OPC in Practice: Bioflavanols and Their Application.* Rome, Italy: Alpha Omega Publishers; 1993.
42. Shield MJ. Anti-inflammatory drugs and their effects on cartilage synthesis and renal function. *Eur J Rheumatol Inflamm.* 1993;13:7–16.
43. Skibola CF, Smith MT. Potential health impacts of excessive flavonoid intake. *Free Radic Biol Med.* 2000;29:375–383.
44. Soil AH, Weinstein VIM, Kurata J, McCarthy D. Nonsteroidal anti-inflammatory drugs and peptic ulcer disease. *Ann Intern Med.* 1991;114:307–319.
45. St Leger AS, Cochrane AL, Moore F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet.* 1979;1:1017–1020.
46. Tixier JM, Godeau G, Robert AM, Hornebeck W. Evidence by in vivo and in vitro studies that binding of pycnogenols to elastin affects its rate of degradation by elastases. *Biochem Pharmacol.* 1984;33:3933–3939.
47. Ueno I, Nakano N, Hirono I. Metabolic fate of [14C] quercetin in the ACI rat. *Jpn J Exp Med.* 1983;53:41–50.
48. Vinson JA. Flavonoids in foods as in vitro and in vivo antioxidants. *Adv Exp Med Biol.* 1998;439:151–164.
49. Whelton A, Hamilton CW. Nonsteroidal anti-inflammatory drugs: effects on kidney function. *J Clin Pharmacol.* 1991;31:588–598.
50. Yochum L, Kushi LH, Meyer K, Folsom AR. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *Am J Epidemiol.* 1999;149:943–949.