

FUTURE PET NUTRITION: JOINT, SKIN & BONE

INGREDIENTS: SCIENTIFIC NUTRITIONAL FACTS

INGREDIENTS OF FPN JOINT, SKIN & BONE

1. Cissus Quadrangularis : 21/100 grams (about 20%).
2. MSM : 21/100 grams.
3. Brewer's Yeast : 21/100 grams.
4. Krill Omega 3 powder : 21/100 grams.
5. Taurine : 11/100 grams (about 10%).
6. Bromelain : 02/100 grams.
7. Pine Bark : 1.25/100 grams.
8. Beef Flavouring : 1.25/100 grams.
9. Vitamin C : 0.375/100 grams.
10. Manganese : 0.125/100 grams.

BASIC INGREDIENT INFORMATION AND RESEARCH

1. CISSUS QUADRANGULARIS (21/100 grams (about 20% of product))

Cissus Quadrangularis (Cissus Q) is a plant of the grape family (vitaceae), which occurs naturally in Limpopo and Mpumalanga provinces, Africa and Asia. Cissus Q has been used in Ayurvedic medicine for more than 5000 years for its joint and bone healing properties. One of its traditional names is “Bone Setter” in reference to its effect in accelerated bone healing.

1.1. Cissus Q strengthens bones, ligaments, tendons and fasciae, increases bone mass, prevents bone loss (anti-osteoporotic effect) and increases the healing speed of fractures and sores.

Osteoblasts are bone-forming cells. Of the three types of bone cells, they are the ones that produce the bone matrix (organic material that makes up bone). The matrix includes molecules such as collagen protein fibers, which gives bone its flexibility, and calcium (Ca²⁺) and phosphate (PO₄⁻) ions, which give bone its rigidity.

Cissus Q promotes the synthesis of Osteoblasts by up to 69%. (Muthusami S, et al **Cissus quadrangularis augments IGF system components in human osteoblast like SaOS-2 cells.** *Growth Horm IGF Res.* (2011))

When dams were treated with Cissus Q, the total length of ossified cartilage (bone) of their pups was significantly higher (P<0.001– 0.0001) than the control group. (**Petroleum Ether Extract of Cissus Quadrangularis (LINN) Stimulates the Growth of Fetal Bone during Intra Uterine Developmental Period: A Morphometric Analysis.**

Bhagath Kumar Potu,^I Muddanna S Rao,^I Gopalan Kutty N,^{II} Kumar MR Bhat,^I Mallikarjuna Rao Chamallamudi,^{II} and Soubhagya Ranjan Nayak)

Tendons, ligaments and fasciae are all made of Collagen. Fasciae are bands or sheets of connective tissue fibers that form beneath the skin to attach, stabilize, enclose, and separate muscles and other internal organs. Cissus Q increases Collagen synthesis in Osteoblast cells by up to 106%.

(Muthusami S, et al **Effects of Cissus quadrangularis on the proliferation, differentiation and matrix mineralization of human osteoblast like SaOS-2 cells.** J Cell Biochem. (2011))

In a study on rats mimicking menopause, Cissus Q has been able to prevent losses in bone strength fully and prevent up to 86% of the losses in bone thickness. (Potu BK, et al **Anti-osteoporotic activity of the petroleum ether extract of Cissus quadrangularis Linn. in ovariectomized Wistar rats.** Chang Gung Med J. (2010)). See also Banu J, et al **Inhibition of Bone Loss by Cissus quadrangularis in Mice: A Preliminary Report** . J Osteoporos. (2012)

1.2. Cissus in an Analgesic (painkiller).

A study of exercised trained men, who experienced chronic joint pain due to exercise noted that Cissus Q supplementation caused a 31% reduction in pain and impairment. (Phys Sportsmed. 2013 Sep;41(3):29-35. doi: 10.3810/psm.2013.09.2021. **Cissus quadrangularis reduces joint pain in exercise-trained men: a pilot study.** Bloomer RJ1, Farney TM, McCarthy CG, Lee SR.)

See also Kumar R, et al **CNS activity of aqueous extract of root of Cissus quadrangularis Linn. (Vitaceae)** . J Diet Suppl. (2010). "Prominent analgesic activity was observed using the hot plate method. The paw licking time was delayed significantly. The extract also displayed prominent smooth muscle relaxant activity. The results suggest that the aqueous extracts of C. quadrangularis roots possess anticonvulsant, analgesic, and smooth muscle relaxant properties."

In other analgesic tests, Cissus Q also provoked a significant reduction of the number of writhes in acetic acid-induced writhing response in mice. Cissus Q also significantly reduced the licking time in both phases of the formalin test. The results suggest peripheral and central analgesic activity of Cissus Q. (Panthong A, et al **Analgesic, anti-inflammatory and venotonic effects of Cissus quadrangularis Linn.** J Ethnopharmacol. (2007)).

Cissus Q also contains some currently unknown tannin-like structures that are potent cyclooxygenase (COX) inhibitors. COX's are chemical messengers that cause the pain and inflammation from injuries, arthritis, etc. (Panthong A, *et al* **Analgesic, anti-inflammatory and venotonic effects of Cissus quadrangularis Linn.** *J Ethnopharmacol.* (2007))

1.3. Cissus Q is an Anti-inflammatory and Anti-oxidant

Edema is the medical term for swelling. It is a general response of the body to injury or inflammation. Edema results whenever small blood vessels become "leaky" and release fluid into nearby tissues. The extra fluid accumulates, causing the tissue to swell.

A test for anti-inflammatory effect is to measure the effect of a substance on edema in a rat's ear, caused by applying ethyl phenylpropiolate. In the acute phase of inflammation Cissus Q elicited an inhibitory effect on the edema formation in the rats' ear induced by ethyl phenylpropiolate, as well as on the formation of the paw edema in rats induced by both carrageenin and arachidonic acid. It is likely that CQ is a dual inhibitor of arachidonic acid metabolism. (Panthong A, *et al* **Analgesic, anti-inflammatory and venotonic effects of Cissus quadrangularis Linn.** *J Ethnopharmacol.* (2007)). Arachidonic acid plays a central role in inflammation related to injury and many diseased states. How it is metabolized in the body dictates its inflammatory or anti-inflammatory activity.

[For clarification sake, arachidonic acid is a non-essential Omega 6 fatty acid. However, it does become essential if there is a deficiency in linoleic acid or if there is an inability to convert linoleic acid to arachidonic acid, which is required by most mammals. Some mammals lack the ability to, or have a very limited capacity to, convert linoleic acid into arachidonic acid, making it an essential part of their diets. Since little or no arachidonic acid is found in common plants, such animals are obligate carnivores. The cat is a common example.]

Cissus Q contains anti-oxidant compounds that activate heme-oxygenase 1 (HO-1), which suppresses inflammation. Cissus Q also contains some currently unknown tannin-like structures that are potent cyclooxygenase

(COX) inhibitors. COX's are chemical messengers that cause the pain and inflammation from injuries, arthritis, etc. (Panthong A, *et al* **Analgesic, anti-inflammatory and venotonic effects of Cissus quadrangularis Linn.** *J Ethnopharmacol.* (2007))

1.4. Cissus Q is an Anti-histamine

Cissus Q has shown to reduce by 44% the early phase swelling (indicative of histamine release) in rats injected with carrageen. (Begum VH, Sadique J **Anti histaminic activity of cissus quadragularis.** *Anc Sci Life.* (1999)

1.5. Cissus Q is an Anti-cortisol

Cissus Q is a glucocorticoid antagonist. In other words it reduces the negative (katabolic) effects of cortisol, the “stress” hormone in mammals.

In the bodies of mammals metabolism consists of anabolic (constructive metabolism) and katabolic (destructive metabolism) activities. For example, if it was not for katabolic activities in the body of a dog or cat, it would literally just keep growing bigger and bigger muscles to gigantic proportions, until it can no longer nutritionally sustain itself and it dies. In fact, the species would probably become extinct.

Cortisol, a so-called glucocorticoid (steroid hormone), is produced from cholesterol by the adrenal glands. It plays a major part in the katabolic processes of the animal body and particularly so as a result of stress. Stress in an animal can be caused by malnutrition from poor quality commercial dog foods, dehydration, cold, illness, injury, abuse, exercise, pregnancy, etc.

Stress induced high Cortisol levels have the following negative effects:

1. Cortisol reduces bone formation, which can promote osteoporosis.
2. Cortisol transports potassium out of cells in exchange for an equal number of sodium ions.
3. Cortisol reduces calcium absorption in the intestine.
4. Cortisol diminishes collagen synthesis.

5. Cortisol raises the free amino acids in the serum. It does this by inhibiting collagen formation, decreasing amino acid uptake by muscle, and inhibiting protein synthesis.
6. Cortisol diminishes the immune system.
7. High levels of stress and increases in cortisol have been found to lengthen wound-healing time in healthy human by up to 40%. (Marucha PT, Kiecolt-Glaser JK, Favagehi M (1998). **"Mucosal wound healing is impaired by examination stress"**. *Psychosom Med* **60** (3): 362–5).

2. MSM

21/100 grams.

Like Glucosamine, MSM acts as a sulphur supplement, which an animal obtains from the amino acids methidione and cysteine.

Sulphur is a component of four amino acids: methionine, cysteine, cystine, and taurine. Sulphur performs a number of important functions, such as providing a place for these amino acids to bond together, thus solidifying a protein structure. It is found in high concentrations in the protein structure of the joints, hair, nails, and skin.

Patients with osteoarthritis of the knee taking MSM for 12 weeks showed an improvement in pain and physical function. (BMC Complement Altern Med. 2011 Jun 27;11:50. doi: 10.1186/1472-6882-11-50. **Efficacy of methylsulfonylmethane supplementation on osteoarthritis of the knee: a randomized controlled study.** Debbi EM1, Agar G, Fichman G, Ziv YB, Kardosh R, Halperin N, Elbaz A, Beer Y, Debi R)

3. Brewer's Yeast 21/100 grams.

Brewer's Yeast is made from a single-celled fungus called *Saccharomyces cerevisiae* is ordinarily associated with beer manufacturing. It is a rich source of minerals, particularly selenium, B-Complex Vitamins and chromium, an essential trace mineral that helps the body maintain normal blood sugar levels.

The B-Complex minerals include B1 (Thiamine), B2 (Riboflavin), B3 (Niacin), B5 (Pantothenic Acid) B6 (Pyridoxine), B9 (Folic Acid) and B7 (Biotin). These Vitamins help break down carbohydrates, fats and proteins into energy, they support the nervous system, help maintain the muscles used in digestion and they keep skin, hair, eyes, mouth and liver healthy.

4. Krill Omega 3 Powder 21/100 grams.

Krill are small, pink and opaque Arctic marine crustaceans that feed on phytoplankton, microscopic, single-celled plants that drift near the ocean's surface and live off carbon dioxide and the sun's rays. The pink colour of krill and the reddish brown colour of krill powder is caused by an aquatic carotenoid called Astaxanthin, which the Krill gets from its diet.

4.1 Source of High Quality 83% Bio-available Protein with Excellent Amino Acid Profile.

4.2 Superior source of Omega 3 and Fatty Acids.

4.3 Excellent Source of Minerals

- Calcium 2.5%
- Phosphorus 1.55%
- Iron 57.2 ppm
- Copper 74 ppm
- Zinc 71.8 ppm
- 71.8 ppm
- Sodium 0.8%
- Potassium 0.4%
- Magnesium 0.4%
- Nickel 2.9 ppm
- Chromium 0.4 ppm
- Manganese 5.4 ppm
- Cobalt 1.4 ppm
- Selenium 12.5 ppm
- Aluminium 65.8 ppm

4.4 Omega 3's, Found Abundantly in Krill are Anti-carcinogenic

The long chain Omega 3 poly-unsaturated fatty acids (n-3 PUFAs) eicosapentaenoic acid (EPA) and its metabolite, docosahexaenoic acid, inhibit cancer formation. (**Omega-3 polyunsaturated fatty acids selectively inhibit growth in neoplastic oral keratinocytes by differentially activating ERK1/2**)

Zacharoula Nikolakopoulou¹↑, Georgios Nteliopoulos², Adina Teodora Michael-Titus³ and Eric Kenneth Parkinson)

4.5 Astaxanthin is a Super Nutrient and the Most Powerful Natural Anti-oxidant on the Planet.

Astaxanthin is the most potent carotenoid antioxidant when it comes to free radical scavenging, 65 times more powerful than vitamin C, 54 times more powerful than beta-carotene, and 14 times more powerful than vitamin E.

Astaxanthin is nature's most powerful anti-oxidant in respect of singlet, oxygen quenching. Exponentially more so than beta-carotene (11 x stronger), alpha lipoic acid (75 x stronger), green tea catechins and Vitamin E (550 x stronger), Coenzyme Q 10 (800 x stronger), Vitamin C (6000 x stronger), etc.

(Carotenoid Science, Vol.11, 2007, 16-20 ISSN 1880-5671 **Quenching Activities of Common Hydrophilic and Lipophilic Antioxidants against Singlet Oxygen Using Chemiluminescence Detection System.** Yasuhiro Nishida*, Eiji Yamashita and Wataru Miki)

“Living organisms possess defense mechanisms against oxidative damage. One of the most important ways is using anti-oxidants, such as ascorbic acid (Vit C), polyphenols, Coenzyme Q10 (CoQ10), tocopherols or carotenoids, for quenching and/or scavenging against reactive oxygen species (ROS).

Singlet oxygen (1O_2) is a non-radical reactive oxygen species (ROS) with one of the strongest activities. It directly damages biological lipids, proteins and DNA, which damage is then related to serious diseases such as diabetes, hypertension and cancer.

The substances that were tested were all common hydrophilic and lipophilic antioxidants such as polyphenols, tocopherols, carotenoids, ascorbic acid, coenzyme Q10 and α -lipoic acid.

Overall, astaxanthin exhibited the most potent singlet oxygen quenching activity among the compounds tested in this study because it showed a stable superior property under the three different conditions.”

See the excellent graph on page 20 of the study below?

<http://www.cyanotech.com/pdfs/bioastin/bat140.pdf>

Astaxanthin crosses the blood-brain barrier and the blood-retinal barrier, whilst beta-carotene and lycopene does not. This brings anti-oxidant and anti-inflammatory protection to eyes, the brain and to the central nervous system.

Astaxanthin is soluble in fats and is therefore incorporated into cell membranes.

4.6 Astaxanthin Improves Skin Elasticity, Skin Aging and Protects Skin from Sun Damage

Astaxanthin comes from algae called *hamotococcus pluvialis* (*h. pluvialis*), which the Krill eats. *H. pluvialis* drifts close to the ocean surface and Astaxanthin protects it from ultra violet radiation that it is constantly exposed to.

Astaxanthin is found in most organs in the body, but it accumulates in the skin, and in all the skin layers. Topical sunscreens can reach only the outermost layers.

Ultraviolet radiation is the most serious environmental risk factor for skin cancer. Exposure of lipids and tissues to light, especially UV-light, can lead to production of singlet oxygen and free radicals and photo-oxidative damage of these lipids and tissues. Excessive exposure of unprotected skin to sunlight results in sunburn and can also lead to photo-induced oxidation, inflammation, immune-suppression, aging and even carcinogenesis of skin cells.

Astaxanthin enables longer uv radiation exposure/reduces the risk of sunburn and, if uv damage does occur, Astaxanthin materially diminishes inflammation, photo immune-suppression, photo aging and the chances of skin cancer development. (Wei Sheng Yan Jiu. 2011 Sep;40(5):551-4. **Protective effects of astaxanthin against oxidative damage induced by 60Co gamma-ray irradiation.** Zhao W1, Jing X, Chen C, Cui J, Yang M, Zhang Z)

See also the very detailed research article called **Haematococcus astaxanthin: applications for human health and nutrition.** Martin Guerin, Mark E. Huntley and Miguel Olaizola below at:

<http://www.cyanotech.com/pdfs/bioastin/bat109.pdf>

See also ***Cosmetic benefits of astaxanthin on humans subjects*** Kumi Tominaga, Nobuko Hongo, Mariko Karato and Eiji Yamashita:

Two human clinical studies were performed. One was an open-label non-controlled study involving 30 healthy female subjects for 8 weeks. Significant improvements were observed by combining 6 mg per day oral supplementation and 2 ml (78.9 µM solution) per day topical application of Astaxanthin. Astaxanthin showed improvements in skin wrinkle (crow's feet at week-8), age spot size (cheek at week-8), elasticity (crow's feet at week-8), skin texture (cheek at week- 4), moisture content of corneocyte layer (cheek in 10 dry skin subjects at week-8) and corneocyte condition (cheek at week-8). It may suggest that Astaxanthin can improve skin condition in all layers such as corneocyte layer, epidermis, basal layer and dermis by combining oral supplementation and topical treatment.

Another study was a randomized double-blind placebo controlled study involving 36 healthy male subjects for 6 weeks. Crow's feet wrinkle and elasticity and trans-epidermal water loss (TEWL) were improved after 6 mg of Astaxanthin (the same as former study) daily supplementation. Moisture content and sebum oil level at the cheek zone showed strong improvement.

4.7 Astaxanthin Boosts the Immune Function

Immune response cells are particularly sensitive to oxidative stress and membrane damage by free radicals because they rely heavily on cell-to-cell

communications via cell membrane receptors.

The phagocytic action (engulfing and absorbing bacteria and other small cells and particles) of some of these cells releases free radicals that can rapidly damage these cells if they are not neutralized by antioxidants such as Astaxanthin.

4.8 Astaxanthin Prevents cancer

Epidemiological studies reveal that dietary intake of Astaxanthin along with other carotenoids is associated with the reduced risk of many different types of cancer.

Unlike many pharmaceuticals, Astaxanthin shows beneficial effects against cancer at each stage of its development:

- It pro-actively prevents cancer from starting by protecting DNA from ultraviolet and oxidant damage.
- It promotes early detection and destruction of cells that have undergone malignant transformation by boosting immune surveillance. (Yuan JP, Peng J, Yin K, Wang JH. **Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae.** *Mol Nutr Food Res.* 2011 Jan;55(1):150-65.)
- It prevents cancerous growth in cells that evade immune detection by reducing inflammatory changes such as those that appear in aging. (Yasui Y, Hosokawa M, Mikami N, Miyashita K, Tanaka T. **Dietary astaxanthin inhibits colitis and colitis-associated colon carcinogenesis in mice via modulation of the inflammatory cytokines.** *Chem Biol Interact.* 2011 Aug 15;193(1):79-87.

Nagendraprabhu P, Sudhandiran G. **Astaxanthin inhibits tumor invasion by decreasing extracellular matrix production and induces apoptosis in experimental rat colon carcinogenesis by modulating the expressions of ERK-2, NFkB and COX-2.** *Invest New Drugs.* 2011 Apr;29(2):207-24.

- Astaxanthin blocks the rapid cell replication of tumors in their growth phase by stopping the cancer cells' reproductive cycle and by restoring cancer cells' ability to die off by apoptosis (normal cellular death in the progress of time).

Palozza P, Torelli C, Boninsegna A, et al. **Growth-inhibitory effects of the astaxanthin-rich alga Haematococcus pluvialis in human colon cancer cells.** *Cancer Lett.* 2009 Sep 28;283(1):108-17.

Song XD, Zhang JJ, Wang MR, Liu WB, Gu XB, Lv CJ. **Astaxanthin induces mitochondria-mediated apoptosis in rat hepatocellular carcinoma CBRH-7919 cells.** *Biol Pharm Bull.* 2011;34(6): 839-44.

Zhang X, Zhao WE, Hu L, Zhao L, Huang J. **Carotenoids inhibit proliferation and regulate expression of peroxisome proliferators-activated receptor gamma (PPARgamma) in K562 cancer cells.** *Arch Biochem Biophys.* 2011 Aug 1;512(1):96-106.

Song X, Wang M, Zhang L, et al. **Changes in cell ultrastructure and inhibition of JAK1/STAT3 signaling pathway in CBRH-7919 cells with astaxanthin.** *Toxicol Mech Methods.* 2012 Nov;22(9): 679-86.

5. Taurine

11/100 grams (about 10%).

Taurine is an amino sulfonic acid, which is found in raw animal and seafood meat tissue. It is highly water soluble and the relatively small amounts of taurine in the pre-processed commercial dog foods is materially degraded by the heat processing of virtually all commercial dog foods. Taurine is virtually absent for most commercial dog foods. Although Taurine can be synthesized by dogs, certain breeds appear to be more susceptible to Taurine deficiency, amongst others, American cocker spaniels and giant breed dogs like the Newfoundland. The ability to synthesize Taurine diminishes with age.

Taurine is generally associated with longevity. Amongst many other properties, it improves blood flow, it is an important indirect antioxidant, it appears to protect the skin after Ultra Violet sun damage and it improves bioavailability of the lipid soluble vitamins A, D, E, K, and F. Amino Acids. 2000;19(2):409-21. **Taurine as a universal carrier of lipid soluble vitamins: a hypothesis.** Petrosian AM1, Haroutounian JE.

“In the literature Taurine is characterized as a non-specific growth or blood clotting factor, an antioxidant, a membrane protector, or a regulator of calcium ion homeostasis, just as vitamins A, D, E, F, and K are similarly characterized. On the basis of recent finding concerning the relationship between taurine and the aldehyde of vitamin A-retinal (Petrosian and Haroutounian, 1988, 1998; Petrosian et al., 1996), as well as on the basis of data from the literature, we now suggest a hypothesis that taurine promotes the bioavailability of the lipid soluble vitamins A, D, E, K, and F, probably by forming different types of water soluble, easily hydrolysable complexes. It is quite possible that the ability of taurine to convert lipids and lipid soluble substances into a water soluble state is the key to understanding the unusually wide diversity of biological phenomena associated with Taurine. This form of delivery may be an additional, secondary mechanism for the transport of lipid soluble vitamins, which was probably acquired early in evolution, and remains extremely important for mammals and humans directly after birth for a variety of physiological functions such as: vision in normal and in emergency situations, rapid blood clotting, sperm eruption, and situations requiring a prompt consumption of lipid soluble vitamins characteristic of excitable

systems. Clearly, the role of taurine in the physiology of the water insoluble vitamins remains an enigma and is worthy of further investigations.”

6. Bromelain 02/100 grams.

Bromelain is a glycoprotein extracted from pineapples.

Bromelain is an effective mucolytic agent (anti-histamine) in respiratory tract diseases.

Bromelain's pharmacological activity is via several mechanisms:

1. Induction of proteolytic activity at inflammatory sites;
2. Activation of fibrinolysis activity via the plasminogen-plasmin system;
3. Depletion of kininogen;
4. Inhibition of pro-inflammatory prostaglandin biosynthesis and initiation of prostaglandinE1 accumulation (which inhibits the release of polymorphonuclear leukocyte lysosomal enzymes).

Bromelain Studies;

1. Ako H, Cheung A., Matsura P. ***Isolation of a fibrinolysis enzyme activator from commercial bromelain.*** Arch Int Pharmacodyna 1981;254:157-167.
2. Taussig S. ***The mechanism of the physiological action of bromelain.*** Med Hypothesis 1980;6:99- 104.
3. Felton G. ***Does kinin released by pineapple stem bromelain stimulate production of prostaglandin E1-like compounds?*** Hawaii Med J 1977;36:39- 47.
4. Kelly GS. ***Bromelain: a literature review and discussion of its therapeutic applications.*** Altern Med Rev 1996;1:243-257.

7. Pine Bark 1.25/100 grams.

Pine Bark is extracted from a tree called Pinus Pinaster or French Maritime Pine.

Pycnogenol is a patented formulation of French Maritime Pine Bark extract, which is standardized to between 65-75% Procyadin compounds per weight.

Procyadins are chain like structures, which consist of catechins similar to that found in green tea, grape seed extract and cocoa extract. Although most of the research is on Pycnogenol, technically, we cannot use the term Pycnogenol, except where it is mentioned specifically in studies, because it is patented. For the sake of this document I will use the term Pine Bark.

7.1 Pine Bark is an Analgesic (painkiller)

In persons with primary grade 1 osteoarthritic symptoms, supplementation of Pine Bark extract at 50mg thrice daily was noted to, over the course of 90 days, reduce pain and functional impairment associated with osteoarthritis in the range of 35-52% (***Pycnogenol supplementation reduces pain and stiffness and improves physical function in adults with knee osteoarthritis.*** Reza Farid^a, Zahra Mirfeizi^a, Mahyar Mirheidari^a, Zahra Rezaieyazdi^a, Hassan Mansouri^a, Habib Esmaili^a, Sherma Zibadi^b, Peter Rohdewald^c, Ronald Ross Watson).

7.2 Pine Bark is a Powerful Anti-oxidant and Anti-inflammatory

“Pycnogenol® has been demonstrated in various studies to be one of the most powerful natural antioxidants. At the University of California, Berkeley, Dr. Lester Packer showed that Pycnogenol® is more powerful than vitamins C and E. Dr. Packer found that Pycnogenol® is so powerful that it recycles oxidized (spent) vitamin C back to the bioactive form and protects vitamin E from oxidation. Furthermore, Dr. Benjamin La (Loma Linda University, CA) discovered that Pycnogenol® stimulates production of antioxidant enzymes inside cells, thus reinforcing their own first defense line against free radicals.”

An in vitro study carried out at the University of Tokyo, Japan, showed that Pycnogenol® is a dramatically more powerful antioxidant than any other

antioxidant tested to protect fragile lipids of the eye. 5 times more potent than grape seed extract, 9 times more potent than Vitamin E and 45 times more potent than CoQ10. (Jpn J Infect Dis. 2008 Jul;61(4):279-85. **Pycnogenol, a procyanidin-rich extract from French maritime pine, inhibits intracellular replication of HIV-1 as well as its binding to host cells.** Feng WY1, Tanaka R, Inagaki Y, Saitoh Y, Chang MO, Amet T, Yamamoto N, Yamaoka S, Yoshinaka Y.)

Pycnogenol® stimulates synthesis of antioxidative enzymes inside cells of the arteries thereby doubling the amount of antioxidative enzymes. (Wei, Z. H., Peng, Q. L. and Lau, B.H. S. (1997) **Pycnogenol® enhances endothelial cell antioxidant defenses.** Redox Report, 3(4): 219-224.)

Pycnogenol® inhibits key triggers of inflammation. (Grimm, T., Chovanova, Z., Muchova, J., Sumegova, K., Liptakova, A., Durackova, Z., Högger, P. (2006) **Inhibition of NF-kappaB activation and MMP-9 secretion by plasma of human volunteers after ingestion of maritime pine bark extract (Pycnogenol®).** Journal of Inflammation, 3: 1, 1-6)

Pycnogenol® inhibits the most important pro-inflammatory enzymes, signaling Pycnogenol's bioavailability. (Schäfer A, Chovanová Z, Muchová J, Sumegová K, Liptáková A, Duracková Z, Högger P. (2005) **Inhibition of COX-1 and COX-2 activity by plasma of human volunteers after ingestion of French maritime pine bark extract (Pycnogenol®).** Biomedicine & Pharmacotherapy 60:5-9.)

Pycnogenol® scavenges superoxide radicals in vitro and inhibits edema in vivo. The anti-inflammatory and free radical scavenging activities are closely correlated. (Blazso, G., Gabor, M., Sibbel, R. and Rohdewald, P. (1994) **Anti-inflammatory and superoxide radical scavenging activities of a procyanidins containing extract from the bark of Pinus pinaster sol. and its fractions.** Pharm. Parmacol. Lett., 3: 217-220.)

The tissue destroying enzymes (matrix metalloproteinases) collagenase, elastase and gelatinase are inhibited in vitro. Release of these enzymes from inflammatory cells is also inhibited by Pycnogenol® and its metabolites. (Grimm, T., Schäfer, A. and Högger, P. (2004) **Antioxidant activity and**

inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (Pycnogenol®). Free Radicals Biology and Medicine, 36(6): 811-822.)

7.3 Joint Health

See attached PYCNOGENOL FOR JOINT HEALTH article from Pycnogenol website:

http://www.pycnogenol.com/uploads/tx_atwresearchlibrary/Pycnogenolforjointhealth.pdf

7.4 Pine Bark is Anti Histamine and Anti-asthma

Pycnogenol® blocks release of histamine from mast cells in vitro to the same extent as the anti-asthmatic drug DNCG. Sharma, S.C., Sharma, S. and Gulati, O.P. (2003)Pycnogenol® inhibits the release of histamine from mast cells.Phytotherapy Research 17: 66-69

Panminerva Med. 2011 Sep;53(3 Suppl 1):57-64.

Pycnogenol® improvements in asthma management. Belcaro G1, Luzzi R, Cesinaro Di Rocco P, Cesarone MR, Dugall M, Feragalli B, Errichi BM, Ippolito E, Grossi MG, Hosoi M, Errichi S, Cornelli U, Ledda A, Gizzi G.

Pycnogenol® reduces asthma symptoms and improves lung function of asthmatic patients in a placebo-controlled, cross-over study. Hosseini, S., Pishnamazi, S., Sadrzadeh, M.H., Farid, F., Farid, R. and Watson, R.R. (2001) Pycnogenol® in the management of asthma. Journal of Medicinal Food, 4 (4): 201-209.

Pycnogenol® improves pulmonary functions and reduces symptoms of asthma in children. Lau, B.H.S., Riesen, S.K., Truong, K.P., Lau, E.W., Rohdewald, P. and Barreta, R.A. (2004)Pycnogenol® as an adjunct in the management of childhood Asthma.Journal of Asthma, Vol. 41 (8), 825-832

7.5 Pine Bark is Anti-UV/Sunburn

Applied topically, Pycnogenol® significantly reduces UVB radiation induced-erythema, the procyanidins are the protecting factors. (Blazso, G., Rohdewald, P., Sibbel, R. and Gabor, M. (1995) **Anti-inflammatory activities of procyanidin-containing extracts from Pinus pinaster sol.** Proceedings of the International Bioflavonoid Symposium, Vienna, Austria, ed. Antus, S., Gabor, M. and Vetschera, K. July 16-19, 1995, pages 231-238.)

Oral administration of Pycnogenol® is able to delay and to reduce skin cancer following UV radiation. (Kyriazi, M., Yova, D., Rallis, M and Lima A. (2005) **Cancer chemopreventive effects of Pinus maritima bark extract on ultraviolet radiation and ultraviolet radiation -7,12 dimethylbenz(a)anthracene induced skin carcinogenesis of hairless mice.** Cancer Letters 237: 234-241.)

Pycnogenol® applied after sunburn inhibits UV-induced suppression of immune system. (Sime, S. and Reeve, V.E. (2004) **Protection from inflammation, immunosuppression and carcinogenesis induced by UV radiation in mice by topical Pycnogenol®.** Photochemistry & Photobiology, 79(2): 193-198)

7.6 Pine Bark Improves Bloodflow, Decreased Chronic Veinous Inefficiency (CVI) and Leg Swelling

In persons with Coronary Artery Disease, Pycnogenol at 200mg daily for 8 weeks is associated with an improvement of blood flow independent of changes in blood pressure. (**Effects of Pycnogenol on endothelial function in patients with stable coronary artery disease: a double-blind, randomized, placebo-controlled, cross-over study.** Enseleit F1, Sudano I, Périat D, Winnik S, Wolfrum M, Flammer AJ, Fröhlich GM, Kaiser P, Hirt A, Haile SR, Krasniqi N, Matter CM, Uhlenhut K, Högger P, Neidhart M, Lüscher TF, Ruschitzka F, Noll G.)

Pycnogenol augments endothelium (the inner lining of blood vessels) dependent vasodilation by increasing Nitric Oxide production. Pycnogenol would be useful for treating various diseases whose pathogenesis involve endothelial dysfunction. (**Pycnogenol, French maritime pine bark extract, augments endothelium-dependent vasodilation in humans.** Nishioka K1,

Hidaka T, Nakamura S, Umemura T, Jitsuiki D, Soga J, Goto C, Chayama K, Yoshizumi M, Higashi Y.

In an open, controlled comparative study 40 patients with diagnosed CVI were treated with 360 mg Pycnogenol per day over a period of 4 weeks. The following parameters were investigated before the start of treatment and after 2 and 4 weeks of treatment: circumference of the lower legs and rating of subjective symptoms (scores) of pain, cramps, night-time swelling, feeling of "heaviness", and reddening of the skin. In addition, blood levels of cholesterol LDL and HDL were determined before and at the end of treatment. Pycnogenol significantly reduced the circumference of the lower limbs and significantly improved subjective symptoms. Furthermore, Pycnogenol significantly decreased cholesterol and LDL values in the blood, whereas HDL remained unaffected. (Phytother Res. 2002 Mar;16 Suppl 1:S1-5. **Comparative study of Venostasin and Pycnogenol in chronic venous insufficiency.** Koch R1.)

7.7 Pine Bark Improves Skin Elasticity, Skin Hydration and Collagen and Hyaluronic Acid Synthesis

Skin Pharmacol Physiol. 2012;25(2):86-92. doi: 10.1159/000335261. Epub 2012 Jan 21. **Pycnogenol® effects on skin elasticity and hydration coincide with increased gene expressions of collagen type I and hyaluronic acid synthase in women.** Marini A1, Grether-Beck S, Jaenicke T, Weber M, Burki C, Formann P, Brenden H, Schönla F, Krutmann J.

"20 healthy postmenopausal women were supplemented with Pycnogenol for 12 weeks. Before, during and after supplementation, their skin condition was assessed (i) by employing non-invasive, biophysical methods including corneometry, cutometry, visioscan and ultrasound analyses and (ii) by taking biopsies and subsequent PCR for gene expression analyses related to extracellular matrix homeostasis.

Pycnogenol supplementation was well tolerated in all volunteers. Pycnogenol significantly improved hydration and elasticity of skin. These effects were most pronounced in women presenting with dry skin conditions prior to the start of supplementation. The skin-physiological improvement was

accompanied by a significant increase in the mRNA expression of hyaluronic acid synthase-1 (HAS-1), an enzyme critically involved in the synthesis of hyaluronic acid, and a noticeable increase in gene expression involved in collagen de novo synthesis.

This study provides skin-physiological and molecular evidence that Pycnogenol supplementation benefits human skin by increasing skin hydration and skin elasticity. These effects are most likely due to an increased synthesis of extracellular matrix molecules such as hyaluronic acid and possibly collagen.”

8. Vitamin C (Vit C)

0.375/100 grams.

8.1 Vit C is an anti-oxidant in skin and diminishes UV damage to skin and possible cancer development

“Vit. C, the most plentiful antioxidant in human skin, forms a part of the complex group of enzymatic and non-enzymatic antioxidants that co-exist to protect the skin from reactive oxygen species (ROS). As Vit. C is water soluble, it functions in the aqueous compartments of the cell.^[4] When the skin is exposed to UV light, ROS such as the superoxide ion, peroxide and singlet oxygen are generated. Vit. C protects the skin from oxidative stress by sequentially donating electrons to neutralize the free radicals. The oxidized forms of Vit. C are relatively non-reactive.^[4] Furthermore, they can be converted back to Vit. C by the enzyme dehydro ascorbic acid reductase in the presence of glutathione. Exposure to UV light reduces the availability of Vit. C in the skin.

As mentioned above, the exposure of skin to UV light generates ROS.^[3] These radicals have a potential to start chain or cascade reactions that damage the cells. The harmful effects of ROS occur as direct chemical alterations of the cellular DNA, the cell membrane and the cellular proteins, including collagen.

Oxidative stress also triggers certain cellular events mediated by transcription factors such as ROS upgrade transcription factor activator protein-1 (AP-1) that increases matrix metalloproteinase (MMP) production, leading to collagen breakdown.^[3] Oxidative stress induces nuclear transcription factor kappa B (NFkB). This produces a number of mediators that contribute to inflammation and skin ageing.^[3] ROS also increase the elastin mRNA level in dermal fibroblasts. This may explain the elastotic changes observed in photoaged skin.^[2]

Antioxidants are necessary for neutralizing the ROS formed due to UV exposure.^[2] It is important to note that Vit. C is equally effective against both UVB (290-320 nm) and UVA (320-400 nm).^[2,5] Repeated small doses of UVA penetrate 30-40-times deeper into the dermis as against UVB, which mostly affects the epidermis. UVA mutates and destroys collagen, elastin, proteoglycans and other dermal cellular structures.^[2] Thus, UVA causes skin ageing and possibly melanoma formation. UVB causes sunburn, ROS,

epidermal mutations and skin cancer. Sunscreens when properly applied prevent UV-induced erythema and thymine dimer mutations that contribute to cutaneous carcinogenesis. However, sunscreens block only 55% of the free radicals produced by UV exposure. Photoageing can be prevented by prevention of UV-induced erythema, sunburn cell formation and inducing collagen repair.[2] To optimize UV protection, it is important to use sunscreens combined with a topical antioxidant.”

8.2 Vit C has powerful anti-inflammatory, collagen synthesis and wound healing properties

As stated earlier, Vit. C inhibits NFκB, which is responsible for the activation of a number of pro-inflammatory cytokines such as TNF-alfa, IL1, IL6 and IL8.[2,3] Therefore, Vit. C has a potential anti-inflammatory activity and can be used in conditions like acne vulgaris and rosacea. It can promote wound healing and prevent post-inflammatory hyperpigmentation.[2,3]

8.3 Vit C is required for proper collagen synthesis

Vit. C is essential for collagen biosynthesis. It has been proposed that Vit. C influences quantitative collagen synthesis in addition to stimulating qualitative changes in the collagen molecule.[2] Vit. C serves as a co-factor for the enzymes prolyl and lysyl hydroxylase, the enzymes that are responsible for stabilizing and cross-linking the collagen molecules.[2] Another mechanism by which Vit. C influences the collagen synthesis is by stimulation of lipid peroxidation, and the product of this process, malondialdehyde, in turn stimulates collagen gene expression.[2]

Vit. C also directly activates the transcription of collagen synthesis and stabilizes procollagen mRNA, thereby regulating collagen synthesis.[2,3] Signs and symptoms of Scurvy, a deficiency disease of Vit. C, are due to impaired collagen synthesis.

8.3 Vit C is an Anti-histamine

Vit C has been found to exert a number of effects on histamine. It appears to prevent the secretion of histamine by white blood cells and increase its

detoxification.²⁷ Histamine levels were found to increase exponentially as ascorbic acid levels in the plasma decreased.²⁸

In a study of the effectiveness of intra- nasal vitamin C, 48 subjects received either ascorbic acid solution (n=27) or placebo (n=21) sprayed into the nose three times daily. After two weeks 74 percent of subjects treated with ascorbate solution were found to have decreased nasal secretions, blockage, and edema.

Vitamin C Studies

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10. Manganese 0.125/100 grams.

10.1 Manganese Promotes the Formation of Cartilage and Bone.

Manganese deficiency results in abnormal skeletal development in a number of animal species. Manganese is the preferred cofactor of enzymes called glycosyltransferases. These enzymes are required for the synthesis of proteoglycans that are needed for the formation of healthy cartilage and bone (7).

10.1 Manganese plays an important role in wound healing

Wound healing is a complex process that requires increased production of collagen. Manganese is required for prolidase, an enzyme that functions to provide the amino acid, proline, for collagen formation in human skin cells (8). A genetic disorder known as prolidase deficiency results in abnormal wound healing among other problems, and is characterized by abnormal manganese metabolism (7). Glycosaminoglycan synthesis, which requires manganese-activated glycosyltransferases, may also play an important role in wound healing (9).

10.3 Manganese is an extremely important anti-oxidant.

Manganese superoxide dismutase (MnSOD) is the principal antioxidant enzyme in the mitochondria. Because mitochondria consume over 90% of the oxygen used by cells, they are especially vulnerable to oxidative stress. The superoxide radical is one of the reactive oxygen species produced in mitochondria during ATP synthesis. MnSOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, which can be reduced to water by other antioxidant enzymes (3).

Manganese Studies

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