

Reactive Species and Antioxidant Markers

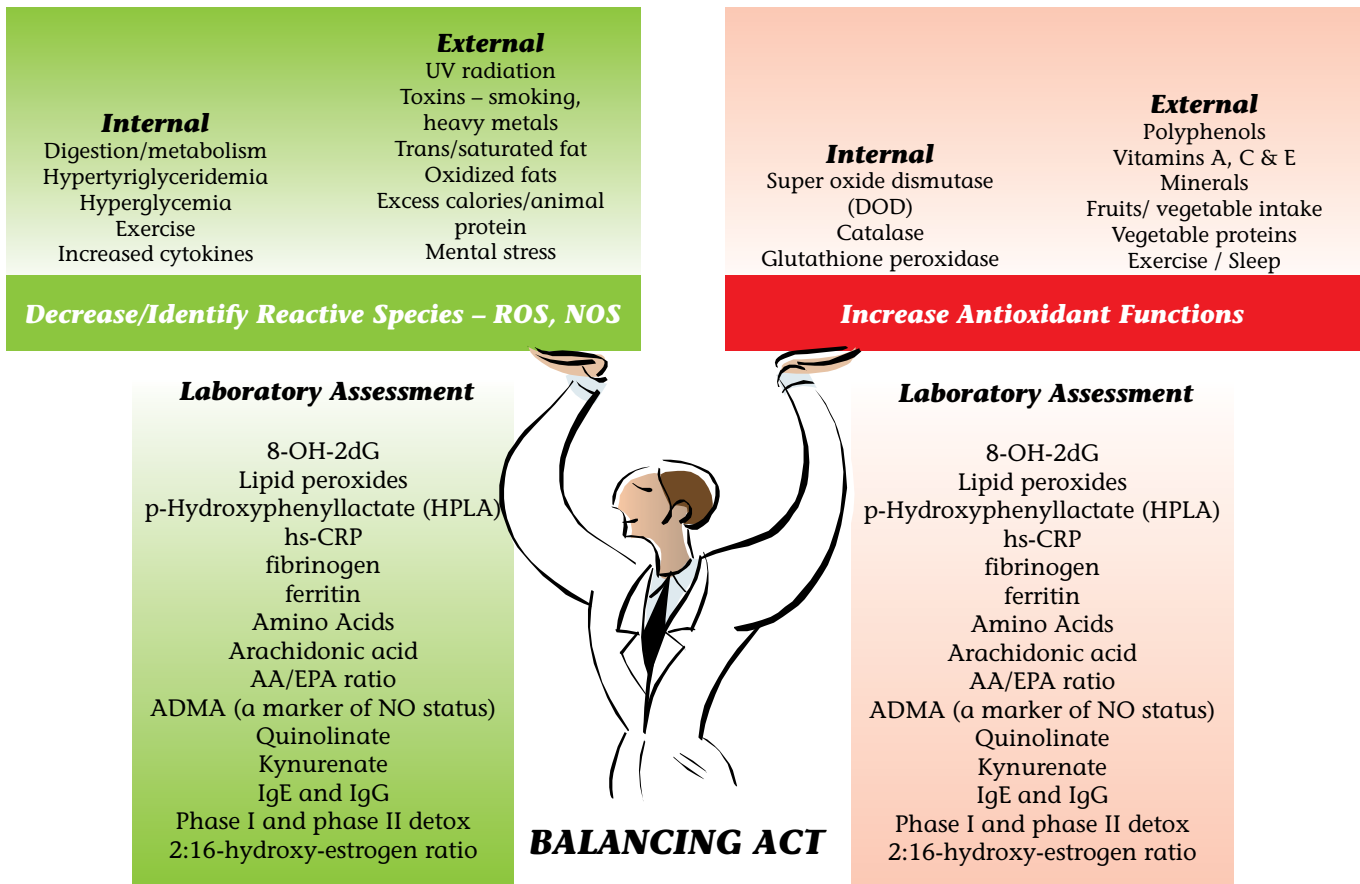
Reactive Species

Normal cellular processes, ultraviolet rays from the sun, environmental pollutants and toxins, excessive intake of protein, total calories or alcohol, and even increased exercise, can increase reactive oxygen (ROS) and reactive nitrogen (RNS) species. These reactive species can also react with other products leading to the production of new reactive species. One example of this is NO reacting with O_2^- (superoxide) producing peroxynitrite (ONOO⁻) which has been shown to damage proteins, lipids and DNA. Damage from ROS and RNS has been suggested as a contributor to chronic diseases such as cancer, atherosclerosis, inflammatory disease, lens tissue disease, Alzheimer disease, and the aging process.¹⁻⁴

Though these reactive species can have adverse effects leading to damaged proteins, lipids, and DNA, they can also be beneficial in low to moderate concentrations. Their positive effects include; functioning in cell signaling reactions, in mitogenic responses, inducing cellular senescence and apoptosis of unwanted cells, and damaging unwanted invaders.⁵ An example of both beneficial and deleterious effects is NO. It is produced by macrophages to defend against invading viruses, bacteria, protozoa.⁶ However, as mentioned above, if the immune system is chronically activated there is a greater chance of peroxynitrite production and increased cellular damage. Therefore, it is crucial that the synthesis of NO, and other reactive species, are under tight regulation.

Cells maintain elaborate mechanisms to contain reactive species and subspecies. These mechanisms are induced when cells are damaged, such as in inflammation, or when there is an increase in oxidative stress. Oxidative stress refers to an imbalance between the production of reactive species and antioxidants or antioxidant defense.⁷ Thus, benefits would be expected from both decreasing reactive species as well as increasing antioxidant abilities. A balance of beneficial effects from low to moderate intakes of ROS and RNS, while ensuring adequate antioxidant capacity to quench those that begin to cause cell damage is essential.

Dietary antioxidants have been found to reduce atherosclerosis in animal and epidemiological research, though there has been some discrepancy in controlled studies.^{8,9} Unfortunately, many study designs often give blanket antioxidant nutrients to all participants without first assessing individual needs. In looking at studies it is important to review the nutrients being evaluated, as well as the type of cellular damage or reactive species. An example of this is that in a recent study of 300 men, nitrotyrosine was associated with fasting glucose in patients with lower intakes of vitamin C and A, but not vitamin E.⁹ If this study had been done looking at just the correlation with vitamin E, they may have erroneously assumed that antioxidants were not correlated. That is why patients should first be tested for individual levels of oxidative stress and inflammation, while also evaluating their antioxidant nutrient status.



Markers of Reactive Species

8-hydroxy-2-deoxyguanosine (8-OHdG)

8-OHdG is a metabolic by-product of oxidative damage by hydroxyl radicals to the guanine bases of DNA, which has the lowest oxidation potential of the four bases.^{10,11} 8-OHdG is both easily formed and is considered mutagenic and carcinogenic.⁵ It is a biomarker of oxidative stress and a potential marker of carcinogenesis. Urinary assessment correlates with the rate of DNA damage and repair, and is a stable product not subject to further metabolism.¹¹⁻¹³ Measurement of urinary 8-OHdG has become a standard bio-marker for evaluating oxidative damage in research studies, and is utilized by clinicians in guiding treatments.¹⁴ 8-OHdG has been used as an assessment of oxidative damage, toxic exposure,^{15, 16, 17} *H pylori*,¹⁸ cancer,^{19, 20, 21} ALS,²² and diabetes.²³ It has also been used as an assessment when evaluating the possible beneficial treatment effects of calcium channel blockers,²⁴ treatments for hypertension,²⁵ cancer treatment,^{10, 26, 27} and dietary interventions.^{28, 29, 30, 31} Numerous studies have indicated that urinary 8-OHdG is not only a biomarker of generalized, cellular oxidative stress but might also be a risk factor for disease states, expanding its importance in research and clinical guidance.^{14, 27} In a study comparing 40 patients with peripheral artery (PAD) disease, to 40 normal controls, serum levels of 8-OHdG were found to be inversely correlated with markers of nitric oxide (a potent vasodilator), as well as walking distance.³²

Lipid Peroxides(TBARS)

Lipid membranes are especially susceptible to oxidative damage because they are the site of greatest exposure to reactive species and they possess high concentrations of molecules that are easily oxidized (unsaturated fatty acids).⁵ Cell membranes are first to encounter molecular oxygen when it is released from hemoglobin, and mitochondrial membranes are the site where molecular oxygen is utilized constantly for ATP production, and where single electron transfer reactions occur.

A serum lipid peroxide level measures the overall effect of oxygen free radical pathology, the risk for degenerative processes, and the need for compensatory anti-oxidant supplementation and/or lifestyle modification. High serum lipid peroxide levels indicate excessive oxygen free radical lipid peroxidation. Malondialdehyde is a major product of lipid peroxidation, it is measured in the thiobarbituric acid reactive substances (TBARS) test. Whenever total antioxidant capacity is inadequate to meet the oxidative challenge, cell membrane oxidation increases, releasing lipid peroxides. Concentrations of lipid peroxides may be measured in urine or serum. Serum has superior sensitivity to slight increases in lipid peroxidation.

p-hydroxyphenyllactate (HPLA)

p-Hydroxyphenyllactate (HPLA) is a metabolite of tyrosine. Unlike other markers that show how much challenge can be tolerated or how much damage has occurred, HPLA reveals how much challenge is being generated within the tissues. Elevated HPLA is associated with tumor growth³³ and leukemia. The methyl ester, methyl-p-hydroxyphenyllactate (MeHPLA), is an important cell growth-inhibiting agent. Tumor cells contain esterase activities that hydrolyze the compound to the free acid, HPLA. Thus, the effects of elevated HPLA may be due to depletion of MeHPLA. Increases in vitamin C have been found effective in lowering p-hydroxyphenyllactic acid.

Quinolate

Chronic stimulation of the immune system causes release of interferon gamma by macrophages. Interferon gamma (IFN- γ) induces increased production of the enzyme, indoleamine-2,3-dioxygenase, that starts the pathway of tryptophan conversion to quinolate. Quinolate can be neurotoxic. Within the brain, the hippocampus is an area rich in NMDA receptors and it is very sensitive to the neurotoxic effects of quinolate. Since the gut is a primary point of chronic inflammatory signal induction via interferon gamma, elevated quinolate may also be indicated inflammatory bowel conditions.

hsCRP

Mild increases in CRP are indicative of low-levels of inflammation which are thought to contribute to heart disease and other chronic conditions. CRP appears to be released as a reaction to endothelial injury. Several important chemotactic factors are released by damaged vascular endothelial cells, including IL-6.³⁴ CRP is then released by the liver to migrate through the plasma, where it is detectable by clinical laboratory methods. High-sensitivity (hs-CRP) assay has been developed to measure chronic, low-grade elevations in CRP.

AA

Essential fatty acids (EFAs), linoleic acid (LA), and alpha-linolenic acid (ALA) are not produced in the body, so they are essential for humans. Arachidonic acid comes from the breakdown of LA and leads to prostaglandins, thromboxanes, leukotrienes, and other oxidized derivatives. Inflammation contributes to a range of acute and chronic conditions that are characterized by the production of these inflammatory cytokines. Omega-3 and omega-6 compete for the same metabolic enzymes, thus the omega-6:omega-3 ratio will significantly influence the ratio of the ensuing eicosanoids (hormones), (e.g. prostaglandins, leukotrienes, thromboxanes etc.), and will alter the body's metabolic function. EFAs are the building blocks for prostaglandins. Elevated fatty acids can also increase the risk of lipid peroxidation.

IL-6

Interleukin-6 (IL-6) is a pro-inflammatory cytokine, or immune protein, that is released in response to infection, burns, trauma, and neoplasia. IL-6 induces the expression of acute phase inflammatory proteins, including fibrinogen and C reactive protein (CRP).³⁵

³⁶ IL-6 induces the liver to manufacture CRP.³⁷ Interleukin 6 (IL-6) has been correlated with disease status and prognosis in cancer patients.³⁸

Antioxidant Support

Antioxidants are molecules which can safely interact with free radicals, and help to diminish their negative effects in the body. There are two types of antioxidants, exogenous (non-enzymatic) and endogenous (enzymatic). Exogenous antioxidants are provided through food or supplements, they scavenge free radicals and work primarily outside of the cell. Endogenous antioxidants are already within the cells and work by inducing antioxidant enzymes. Valko et. al. (2006), have stated that a good antioxidant should meet the following criteria: (1) *specifically quench free radicals*, (2) *chelate redox metals*, (3) *regenerate other antioxidants*, (4) *positively effect on gene expression*, (5) *be readily absorbed*, (6) *have a concentration in tissues and biofluids at a physiologically relevant level*, and (7) *work in both the aqueous and/or membrane domains*.

Exogenous (or non-enzymatic) antioxidants

Some non-enzymatic antioxidants include vitamin C and E, carotenoids, lipoic acid, and flavonoids, polyphenols and minerals. These compounds provide a protection against damage from reactive species. Where and how these antioxidants interact in the body is individual to each antioxidant, and can play a significant role in which antioxidant is needed in each individual. Oxygen free radicals are normal components of mitochondrial membranes and are a problem only when they are formed in an uncontrolled fashion. Similarly, if antioxidants, like vitamins C and E, are consumed in amounts out of proportion with the total spectrum of electron acceptors, the antioxidants can become a part of the problem and may act as pro-oxidants.³⁹

Endogenous (enzymatic) antioxidants

There are several endogenous antioxidant systems within the body. These systems refer to enzymes such as, superoxide dismutase (SOD), glutathione peroxidase and catalase.⁴⁰ Ensuring the minerals required for the formation of metalloenzymes are in adequate supply can ensure antioxidant functions are not impaired. Insufficient copper or zinc could lead to reduced production of zinc-copper superoxide dismutase (SOD).⁴¹ There is considerable research of compounds that increase endogenous antioxidants.⁴² These compounds are primarily botanicals and include, green tea, tumeric, and milk thistle.⁴²

Markers of Antioxidant Capacity

Glutathione

The disulfide chemical group in glutathione makes a particularly suitable, soft landing point for electrons that cause damage if left associated with reactive oxygen molecules. This chemical uniqueness allows understanding of why glutathione is a universal cell reduction-oxidation balancing molecule. Markers of glutathione adequacy include alpha-hydroxybutyrate, pyroglutamate and sulfate. Increased alpha-hydroxybutyrate (alpha-HB) excretion may be related to increased rates of hepatic glutathione synthesis from methionine. Possibly the most widely applicable concept for the interpretation of elevated urinary alpha-HB is increased cytoplasmic NADH2/NAD ratio. Small amounts of pyroglutamate are always present in overnight urine because it is produced as an intermediate in a cycle used in the active transport of amino acids in renal tubules. This process utilizes glutathione as a carrier. When the cycle is impaired, the glutamic acid portion of glutathione is converted to pyroglutamate, which is excreted. This shunt pathway conserves amino acids at the expense of glutathione. Up to one third of the glutathione circulating in blood may be used in this amino acid recovery process. The sulfation pathway is used in Phase II liver detoxification. N-acetylcysteine (NAC) is an effective oral agent for rebuilding total body glutathione, and oral taurine spares sulfur amino acids while providing an effective antioxidant.

Vitamins A, C, and E and β -Carotene

Each of these nutrients plays a critical role in the removal of reactive species. Vitamin A and its precursor, beta-carotene, have independent action in this process, and vitamin E likewise operates with redox potentials uniquely beneficial to specific tissues. Vitamins C and E are major players in antioxidant protection.

Testing fat-soluble vitamins in serum provides direct concentration measures for vitamins A and E, along with beta-carotene. These are the principal molecules examined for deficiencies to reveal need for augmenting dietary intake.

Copper, Manganese, Selenium, Zinc, and Riboflavin

Copper, manganese, selenium, zinc, and riboflavin are considered antioxidant nutrients because they play specific roles as cofactors for the enzymes that catalyze reactions that remove oxygen radicals. These nutrients are cofactors (or precursor vitamins) for the enzymes glutathione reductase (FAD),⁴³ glutathione peroxidase (Se),⁴⁴ and superoxide dismutase (Cu, Mn, Zn).⁴⁵ Total body selenium is so

largely dedicated to this role that some studies have evaluated overall oxidative protection by measuring serum and urinary selenium along with red cell enzymes.⁴⁶

It is important to remember that general healthy lifestyle factors can have a significant impact on overall health. Hyperlipidemia and hyperglycemia, as well as other conditions, can increase oxidative stress. Interleukin 6 was found to be significantly greater in those who had missed a night of sleep, compared to those with uninterrupted sleep.⁴⁷ Many dietary deficiencies can lead to cell damage.^{41, 48} At the same time healthy living may not always be enough to overcome the stress of reactive species from external exposure, disease development or metabolic insufficiencies, and an assessment of antioxidants may be of help to metabolically sure up the cellular fight for survival or thwart the development of a more serious condition.

References

1. Selvaraj N, Bobby Z, Sathiyapriya V. Effect of lipid peroxides and antioxidants on glycation of hemoglobin: an in vitro study on human erythrocytes. *Clin Chim Acta*. Apr 2006;366(1-2):190-195.
2. Yagi K. Lipid peroxides and related radicals in clinical medicine. *Adv Exp Med Biol*. 1994;366:1-15.
3. Kennedy AL, Lyons TJ. Glycation, oxidation, and lipoxidation in the development of diabetic complications. *Metabolism*. Dec 1997;46(12 Suppl 1):14-21.
4. Miyata T, Wada Y, Cai Z, et al. Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. *Kidney Int*. Apr 1997;51(4):1170-1181.
5. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact*. Mar 10 2006;160(1):1-40.
6. Liew FY, Wei XQ, Proudfoot L. Cytokines and nitric oxide as effector molecules against parasitic infections. *Philos Trans R Soc Lond B Biol Sci*. Sep 29 1997;352(1359):1311-1315.
7. Sies H. Role of reactive oxygen species in biological processes. *Klin Wochenschr*. Dec 15 1991;69(21-23):965-968.
8. Cherubini A, Vigna GB, Zuliani G, Ruggiero C, Senin U, Fellin R. Role of antioxidants in atherosclerosis: epidemiological and clinical update. *Curr Pharm Des*. 2005;11(16):2017-2032.
9. Bo S, Gambino R, Guidi S, et al. Plasma nitrotyrosine levels, antioxidant vitamins and hyperglycaemia. *Diabet Med*. Sep 2005;22(9):1185-1189.
10. Erhola M, Toyokuni S, Okada K, et al. Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2'-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. *FEBS Lett*. Jun 9 1997;409(2):287-291.
11. Peoples MC, Karnes HT. Recent developments in analytical methodology for 8-hydroxy-2'-deoxyguanosine and related compounds. *J Chromatogr B Analyt Technol Biomed Life Sci*. Nov 15 2005;827(1):5-15.
12. Shigenaga MK, Ames BN. Assays for 8-hydroxy-2'-deoxyguanosine: a biomarker of in vivo oxidative DNA damage. *Free Radic Biol Med*. 1991;10(3-4):211-216.
13. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*. Dec 1992;13(12):2241-2247.
14. Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetes. *Clin Chim Acta*. Jan 2004;339(1-2):1-9.
15. Yamauchi H, Aminaka Y, Yoshida K, Sun G, Pi J, Waalkes MP. Evaluation of DNA damage in patients with arsenic poisoning: urinary 8-hydroxydeoxyguanine. *Toxicol Appl Pharmacol*. Aug 1 2004;198(3):291-296.
16. Kubota R, Kunito T, Agusa T, et al. Urinary 8-hydroxy-2'-deoxyguanosine in inhabitants chronically exposed to arsenic in groundwater in Cambodia. *J Environ Monit*. Feb 2006;8(2):293-299.
17. Wong RH, Kuo CY, Hsu ML, et al. Increased levels of 8-hydroxy-2'-deoxyguanosine attributable to carcinogenic metal exposure among schoolchildren. *Environ Health Perspect*. Oct 2005;113(10):1386-1390.
18. Baik SC, Youn HS, Chung MH, et al. Increased oxidative DNA damage in Helicobacter pylori-infected human gastric mucosa. *Cancer Res*. Mar 15 1996;56(6):1279-1282.
19. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res*. Dec 1997;387(3):147-163.
20. Bancel B, Esteve J, Souquet JC, Toyokuni S, Ohshima H, Pignatelli B. Differences in oxidative stress dependence between gastric adenocarcinoma subtypes. *World J Gastroenterol*. Feb 21 2006;12(7):1005-1012.
21. Chiou CC, Chang PY, Chan EC, Wu TL, Tsao KC, Wu JT. Urinary 8-hydroxydeoxyguanosine and its analogs as DNA marker of oxidative stress: development of an ELISA and measurement in both bladder and prostate cancers. *Clin Chim Acta*. Aug 2003;334(1-2):87-94.
22. Bogdanov M, Brown RH, Matson W, et al. Increased oxidative damage to DNA in ALS patients. *Free Radic Biol Med*. Oct 1 2000;29(7):652-658.
23. Hata I, Kaji M, Hirano S, Shigematsu Y, Tsukahara H, Mayumi M. Urinary oxidative stress markers in young patients with type 1 diabetes. *Pediatr Int*. Feb 2006;48(1):58-61.
24. Oshima T, Ozono R, Yano Y, et al. Beneficial effect of T-type calcium channel blockers on endothelial function in patients with essential hypertension. *Hypertens Res*. Nov 2005;28(11):889-894.
25. Dhawan V, Jain S. Garlic supplementation prevents oxidative DNA damage in essential hypertension. *Mol Cell Biochem*. Jul 2005;275(1-2):85-94.
26. Mei S, Yao Q, Wu C, Xu G. Determination of urinary 8-hydroxy-2'-deoxyguanosine by two approaches-capillary electrophoresis and GC/MS: an assay for in vivo oxidative DNA damage in cancer patients. *J Chromatogr B Analyt Technol Biomed Life Sci*. Nov 15 2005;827(1):83-87.
27. Matsui A, Ikeda T, Enomoto K, et al. Increased formation of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. *Cancer Lett*. Apr 3 2000;151(1):87-95.
28. Ryan-Borchers TA, Park JS, Chew BP, McGuire MK, Fournier LR, Beerman KA. Soy isoflavones modulate immune function in healthy postmenopausal women. *Am J Clin Nutr*. May 2006;83(5):1118-1125.
29. Shoji H, Franke C, Campoy C, Rivero M. Effect of docosahexaenoic acid and eicosapentaenoic acid supplementation on oxidative stress levels during pregnancy. *Free Radical Researc*. April, 2006 2006;40(4):379-384.
30. Thompson HJ, Heimendinger J, Gillette C, et al. In vivo investigation of changes in biomarkers of oxidative stress induced by plant food rich diets. *J Agric Food Chem*. Jul 27 2005;53(15):6126-6132.
31. Schulpius KH, Papassotiropoulos I, Tsakiris S. 8-hydroxy-2'-deoxyguanosine serum concentrations as a marker of DNA damage in patients with classical galactosaemia. *Acta Paediatr*. Feb 2006;95(2):164-169.
32. Lorenzo Loffredo, Pasquale Pignatelli, Cangemi R. Imbalance between nitric oxide generation and oxidative stress in patients with peripheral arterial disease: effect of an antioxidant treatment. *J Vasc Surg*. 2006;44(3):525-530.
33. Ishiwata K, Vaalburg W, Elsinga PH, Paans AM, Woldring MG. Metabolic studies with L-[1-14C]tyrosine for the investigation of a kinetic model to measure protein synthesis rates with PET. *J Nucl Med*. Apr 1988;29(4):524-529.
34. Verma S, Li SH, Badiwala MV, et al. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation*. Apr 23 2002;105(16):1890-1896.
35. Dalmon J, Laurent M, Courtois G. The human beta fibrinogen promoter contains a hepatocyte nuclear factor 1-dependent interleukin-6-responsive element. *Mol Cell Biol*. Feb 1993;13(2):1183-1193.
36. Ramji DP, Vitelli A, Tronche F, Cortese R, Ciliberto G. The two C/EBP isoforms, IL-6DBP/NF-IL6 and C/EBP delta/NF-IL6 beta, are induced by IL-6 to promote acute phase gene transcription via different mechanisms. *Nucleic Acids Res*. Jan 25 1993;21(2):289-294.
37. Rattazzi M, Puato M, Faggini E, Bertipaglia B, Zambon A, Paultet P. C-reactive protein and interleukin-6 in vascular disease: culprits or passive bystanders? *J Hypertens*. Oct 2003;21(10):1787-1803.
38. Oka M, Yamamoto K, Takahashi M, et al. Relationship between serum levels of interleukin 6, various disease parameters and malnutrition in patients with esophageal squamous cell carcinoma. *Cancer Res*. Jun 15 1996;56(12):2776-2780.
39. Pearson P, Lewis SA, Britton J, Young IS, Fogarty A. The pro-oxidant activity of high-dose vitamin E supplements in vivo. *BioDrugs*. 2006;20(5):271-273.
40. Dong MH, Kaunitz JD. Gastrointestinal mucosal defense. *Curr Opin Gastroenterol*. Nov 2006;22(6):599-606.
41. Ames BN, Atamna H, Killilea DW. Mineral and vitamin deficiencies can accelerate the mitochondrial decay of aging. *Mol Aspects Med*. Aug-Oct 2005;26(4-5):363-378.
42. Nelson SK, Bose SK, Grunwald GK, Myhill P, McCord JM. The induction of human superoxide dismutase and catalase in vivo: a fundamentally new approach to antioxidant therapy. *Free Radic Biol Med*. Jan 15 2006;40(2):341-347.
43. Nuttall KL. Elemental selenium and glutathione reductase. *Med Hypotheses*. Feb 1985;16(2):155-158.
44. Hatanaka N, Nakaden H, Yamamoto Y, Matsuo S, Fujikawa T, Matsusue S. Selenium kinetics and changes in glutathione peroxidase activities in patients receiving long-term parenteral nutrition and effects of supplementation with selenite. *Nutrition*. Jan 2000;16(1):22-26.
45. Neve J, Sinet PM, Molle L, Nicole A. Selenium, zinc and copper in Down's syndrome (trisomy 21): blood levels and relations with glutathione peroxidase and superoxide dismutase. *Clin Chim Acta*. Sep 30 1983;133(2):209-214.
46. Fenech AG, Ellul-Micallef R. Selenium, glutathione peroxidase and superoxide dismutase in maltese asthmatic patients: effect of glucocorticoid administration. *Pulm Pharmacol Ther*. 1998;11(4):301-308.
47. Irwin MR, Wang M, Campomayor CO, Collado-Hidalgo A, Cole S. Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Arch Intern Med*. Sep 18 2006;166(16):1756-1762.
48. Ames BN. Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc Natl Acad Sci U S A*. Nov 13 2006.