

CLINICAL NUTRITION – SOME PERSPECTIVES

Beldeu Singh

The importance of clinical nutrition can be appreciated from the fact that malnutrition is associated with mineral or vitamin or other antioxidant deficiencies and the body ages at an accelerated rate. Even in primary school we were already introduced to the facts relating vitamin deficiencies and diseases like scurvy and beri-beri. The table below lists the large number of diseases caused by or associated with vitamin deficiencies.

TABLE 1: Table of Vitamin Deficiencies

Vitamin	Deficiency Diseases/Conditions
A	Brittle finger nails, cirrhosis of the liver, corneal ulcers, diarrhea, rough, dry, or prematurely aged skin, frequent fatigue, lack of tear secretion, loss of a sense of smell, loss of appetite, night blindness, obstruction of the bile ducts, poor bone growth, skin blemishes, softening of bones and teeth, sties in the eye, susceptibility to respiratory infections, ulcerative colitis, weak tooth enamel, weight loss, xerosis. Carcinogens remain more active when there is a vitamin A deficiency.
B1	Appetite loss, beriberi, constipation, depression, difficulty concentrating, enlarged heart, fatigue, gastrointestinal disorders, hypersensitivity, memory loss, mental confusion, muscle weakness or paralysis, nausea, polyneuritis, rapid heartbeat, vomiting. Eating sugar, cigarette smoke and alcohol depletes thiamine in the body.
B2	Riboflavin deficiency's include baldness, burning of the eyes, cataracts, cracks and sores in the corners of the mouth, depression., dermatitis, digestive disturbances, dilation of pupils, dizziness, dropsy, eye fatigue, feeling of grit or sand on the inside of the eyelids, hair loss, impaired lactation, inability to urinate, inflammation of the tongue and lips, insomnia, lack of stamina and vigor, lesions of the lips, oily skin, red sore tongue, scaling around the nose, mouth, forehead, and ears, sensitivity to light, sluggishness, tension, trembling, vaginal itching, weight loss.

B3	<p>Niacin deficiency produces pellagra (a skin disease), redness of skin and inflammation in the gastrointestinal tract. Impaired short term memory, apprehension, canker sores, dementia, depression, dermatitis, diarrhea, dizziness, emotional instability, general fatigue, halitosis, headaches, hyperirritability, indigestion, insomnia, irritation and inflammation of the mucous membranes of the mouth and gastrointestinal tract, loss of appetite, muscular weakness, nausea, nervous disorders, rashes, rough inflamed skin, strain, swollen red tongue, tender gums, tension, tremors, ulcers, various skin eruptions, vomiting. Excessive consumption of sugar, starches, and antibiotics will deplete the body's supply of niacin.</p>
B5	<p>Pantothenic acid deficiencies are rare but some of the signs are abdominal pains, adrenal exhaustion, burning feet, decreased antibody formation, duodenal ulcers, low blood sugar (hypoglycemia), muscle cramps, restlessness, sensitivity to insulin, skin disorders, upper respiratory infections, vomiting. Deficiencies may occur when the body lacks the intestinal flora needed to synthesize pantothenic acid. A diminished function of the adrenal gland - leads to physical and mental depression. Insufficient secretion of HCl in the stomach leads to poor digestion.</p>
B6	<p>Alzheimer's disease, anemia, anxiety, arthritis, atherosclerosis, B6 deficiency leads to decreased levels of dopamine, confusion, cracks around the mouth and eyes, deficiency is most common in heavy drinkers and users of oral contraceptives, dementia, diminished sex drive, discoloration of the tongue, emotional depression, hair loss, heart disorders involving nerves, hyperactivity, increase in urination, insomnia, irritability, kidney stones, low blood sugar, low glucose tolerance, memory loss, mental depression, mental slowness, neuritis, nervousness, numbness and cramps in arms and legs, sensitivity to insulin, skin lesions, slow learning, temporary paralysis of a limb, visual disturbances, water retention during pregnancy, weakness. Low levels of B6 prevent the proper breakdown of methionine into cystathionine, instead methionine is broken down into homocysteine which attacks heart muscle and allows the deposition of cholesterol around the heart muscle (excess homocysteine may cause atherosclerosis). Smoking reduces B6 levels.</p>

B12	<p>Result from a lack of "intrinsic factor", a mucoprotein enzyme in the gastrointestinal tract, from fish tapeworm infestation, or excessive bacteria in the stomach and intestines. Appetite loss, bleeding gums, brain damage resembling schizophrenia, confusion, depression, difficulty in walking and speaking, difficulty walking, diminished reflex response and sensory perception, fatigue, feeling of deadness, headache, jerking of the limbs, menstrual disorders, nausea, nervousness, neuritis, numbness or stiffness, pale gums, lips and tongue, pins-and-needles or hot-or-cold sensations, poor memory, shooting pains, shortness of breath, sore mouth, sore tongue, soreness and weakness in the legs and arms, unpleasant body odor, weight loss, yellow eyes and skin. Vegetarians are usually deficient in vitamin B12. Laxative reduces the levels of vitamin B12.</p>
C	<p>Vitamin C deficiencies are characterized by anemia, bleeding gums, breaks in capillary walls, emotional disturbances, heart attack and stroke caused by clot formation in capillaries, impaired digestion, loose teeth, lowered resistance to infections, nosebleeds, poor lactation, scurvy, shortness of breath, slow healing of wounds or fractures, swollen and painful joints, tendency to bruise, weakened enamel or dentine.</p>
D	<p>Vitamin D deficiencies are characterized in children with rickets which is stunted bone growth, bowed legs, poorly formed teeth. In adults, deficiencies are characterized by osteomalacia which is softening of bones, causing deformity and fractures. Other signs of deficiency are inadequate absorption of calcium from intestinal tract and retention of phosphorus in the kidney's leading to faulty mineralization of bone structures. Tetany is a condition characterized by muscular numbness, tingling, spasms, and myopia.</p>

E	Vitamin E deficiencies are a rupture of RBC's resulting from their increases fragility, reduction of membrane stability and a shrinkage in collagen may result in a tendency toward muscular wasting or abnormal fat deposits in the muscles and an increased demand for oxygen. Without sufficient E in the body, the essential fatty acids are altered so that blood cells break down and hemoglobin formation is impaired. Several amino acids cannot be utilized, and pituitary and adrenal glands reduce their level of functioning, iron absorption and hemoglobin formation are impaired. A severe deficiency can cause damage to liver and kidneys. In gastrointestinal disease, a prolonged deficiency can cause faulty absorption of fat and of fat-soluble vitamins, possibly resulting in cystic fibrosis, blockage of bile ducts and chronic inflammation of the pancreas. Serious deficiencies in men can lead to degeneration of tissues in the testes possibly leading to sterility. Women severely deficient in E cannot carry a pregnancy term successfully and often are accompanied by miscarriages. Hemorrhaging can occur in newborn infants who lack vitamin E, and blood cells of E deficient babies are prone to weakness (hemolysis). Deficiencies can result in nephritis caused by kidney tubules plugged up with dead cells so urine cannot pass.
K	Vitamin K deficiencies are rare, except for newborns. Fatal intracranial hemorrhage is a risk to newborns that do not receive a vitamin K injection at birth.

Source: nutritionfocus.com, 2005

We also know that taking excess of vitamins for a prolonged period causes health problems as well – a condition called vitaminosis and can be reversed by reducing the intake of vitamins. So, there came about the notion of Recommended Dietary Allowance (RDA) which is based on the idea of preventing deficiency diseases.

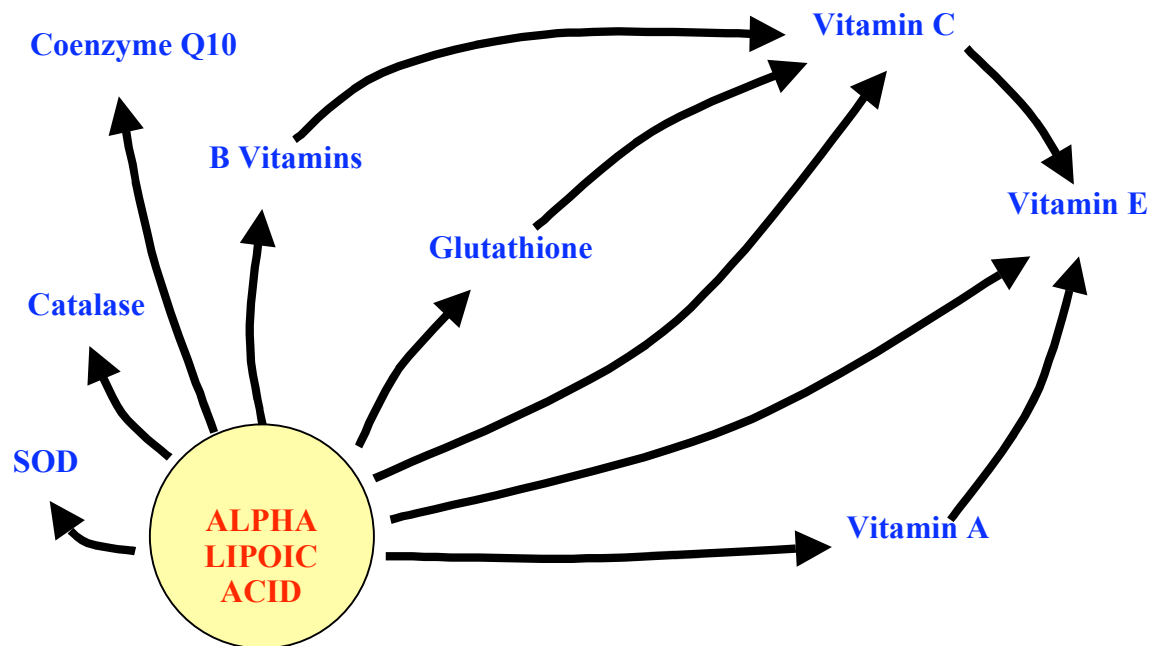
There is a growing body of information based on antioxidant research and how the free radical mechanism generates oxidative stress in tissues leading to inflammations or oxidative injury or inactivation of enzymes involved in cellular function and how free radicals, being extremely reactive, damage protein or hormone molecules or cell membranes and promote aging of the body and how free radicals create the onset of chronic or degenerative conditions especially when they are in excess of antioxidants. This lends support to the new wave on the use of exogenous antioxidants and minerals for maintaining optimal health and to create a vibrant, healthy and productive middle age and to push into the eighties.

There is also a growing focus on the need to research the dietary allowance of antioxidant supplements to prevent and reverse inflammations and chronic diseases or to reverse degenerative diseases and perhaps to boost the natural antioxidant defense mechanism and the immune system in the clinical treatment of diseases. Antioxidants are

known to reduce the incidence of cancers, cardiovascular disease and diabetes but there is little work on therapeutic doses in such conditions or the efficiencies of high doses of antioxidants for a few days in disease states. Perhaps, one approach in the latter is the possibility of taking 3 – 4 times the RDA, depending on age for about four days followed by a “maintenance dose”. This is based on the principle of reversing oxidative stress but the key lies in understanding how the body’s anti-oxidants work in an integrated fashion with exogenous antioxidants in an integrated network.

The antioxidant defense mechanism is a system that comprises the selenium based antioxidants produced in the body that function together with the exogenous antioxidants of plant origin such as vitamin A, B vitamins, vitamin C and vitamin E. Hence administering one vitamin for a specific cure as in the case of drug administration may not be the correct or proper approach for improving total health. The antioxidant approach requires that the antioxidant defense mechanism functions effectively as a system to restore health or improve the body’s ability to fight infection or reverse a chronic disease or to slow down the progress of diseases. And this requires a basic understanding of the lipoic acid antioxidant network and the need for selenium by the body to produce glutathione and selenoproteins.

FIG. 1: ANTIOXIDANTS FUNCTION IN AN INTEGRATED SYSTEM



The endogenous antioxidant system in the mammalian body works in an integrated fashion incorporating the exogenous antioxidants (including vitamins) obtained from plant sources.

Alpha-lipoic acid can be considered the master antioxidant because it readily donates electrons to the antioxidants involved in the Krebs cycle and in mitochondrial metabolic activity. Once coenzyme Q10, glutathione and other vitamins have given away their electrons to neutralize free radicals, they become spent or degraded and lipoic acid can “recharge” them by giving electrons to them. Lipoic acid perhaps recharges all the vitamins in the body and is a crucial complement in the antioxidant network.

Alpha-lipoic acid readily neutralizes the highly reactive hydroxyl and singlet oxygen free radicals thus protecting other antioxidants in aerobic cellular respiration and promotes the efficient functioning of the Krebs cycle.

Understanding the antioxidant network is of fundamental importance in clinical nutrition because it is important to restore the proper and integrated functioning of the antioxidant network for cellular efficiency and energy output as a way to restore health.

Vitamin C is of special importance in health and is “rechargeable” by many antioxidants in the network. It is found in large amounts in nervous tissue, lungs, eye lens and T4 cells. Even the brain can resort to vitamin C from nervous tissue if it comes under oxidative stress. Vitamin C is essential in the formation of collagen – a very important protein produced in the human body and it is used to produce the cementing substance in bone.

Another important perspective in clinical nutrition lies in the association of health with the antioxidant levels in the blood and the declining levels with age. Deterioration in the healthy state begins with excess free radicals over antioxidants to scavenge them and this weakening of the antioxidant defense mechanism suppresses the immune system and opens up the body to infections and/or chronic illnesses. “The antioxidant status of an individual could be important in determining frequency of age-dependent diseases and duration of general health maintenance.”¹ In fact the antioxidant status will become an important tool in clinical nutrition in the near future.

There appears to be a clash between the clinical antioxidant approach to health and the allopathic or drug approach that is more aligned to a specific-cure based on the fact that almost all drugs in current allopathy are immunosuppressive or immunotoxic due to their free radical generating capacity or in other words they deplete antioxidants in the body. The only way to harmonize these two systems is to quickly restore the antioxidant blood levels and to restore the healthy functioning of the antioxidant networks and thereby restore the efficiency of the Krebs cycle in order to prevent a decline in cellular function and depression of the immune system. In essence, this means that the antioxidant defense mechanism must be restored as quickly as possible after the use of allopathic drugs.

Until researchers find better ways to use antioxidants to fight infections and treat crisis situations or find ways to develop rapidly acting antioxidant formulations, allopathic drugs will continue to be used and natural antioxidants will become incorporated in the practice of medicine. Translating ayurvedic information for use in modern therapies is another way to bring the benefits of ayurvedic preparations in new forms in clinical nutrition.

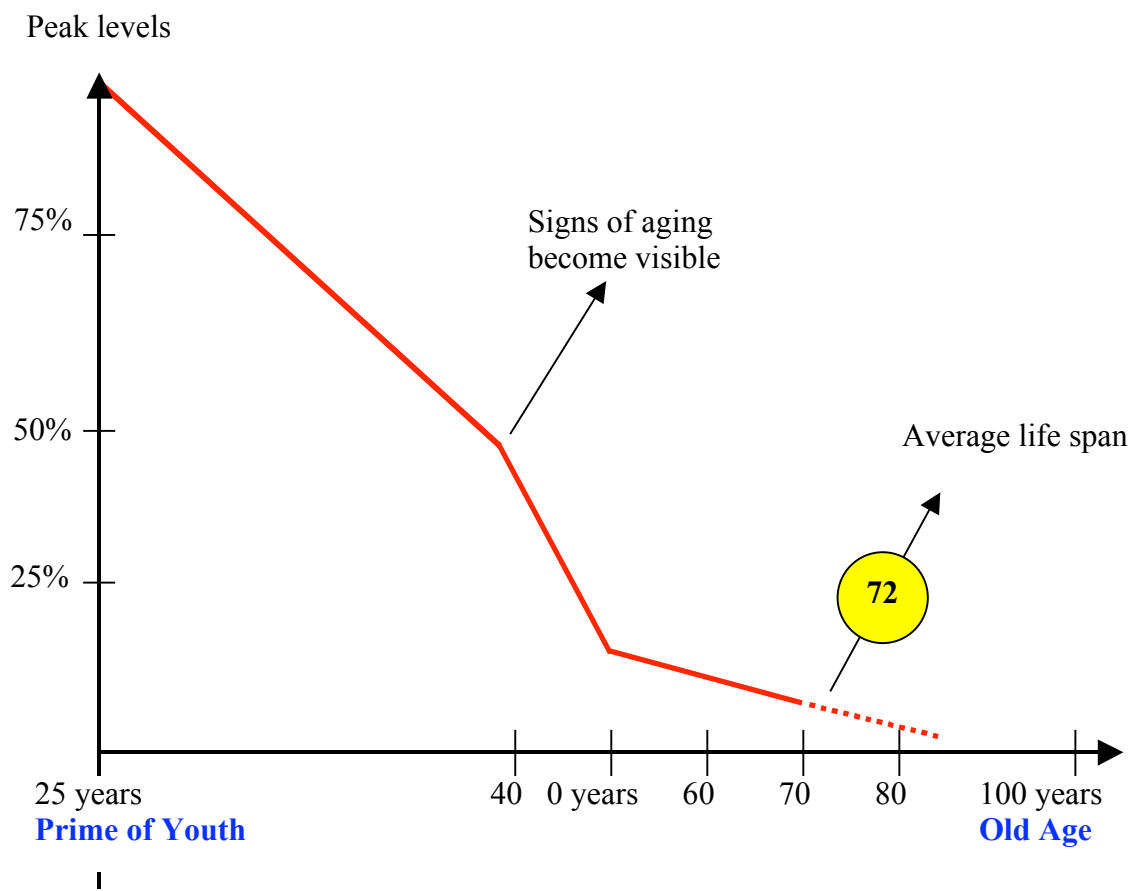
A pertinent piece of information in clinical nutrition is the declining levels of blood antioxidant levels as humans age. The antioxidant blood levels begin to decline from its

peak levels generally associated with prime of youth and seem to crash after the age of 40. This period of decline in the blood antioxidant levels is associated with the observation and findings of higher incidences of degenerative or chronic illnesses. Low levels of antioxidant intake is also linked in studies of groups of people with higher incidence of cardiovascular disease or cancers and this findings are corroborated by studies of populations that consume more antioxidants such as vitamin C and vitamin E who appear to be at a low risk to cardiovascular disease and cancers.

The RDA may be suitable doses for healthy people or the young age groups, below 30 years of age who have relatively high blood antioxidant levels and require only maintenance doses. Severe depletion in vitamins can cause disturbances in the normal functioning of the Krebs cycle and energy utilization in cells.

The link between the destruction of antioxidant system in the healthy functioning of the Krebs cycle and cellular function must be fully appreciated together with the blood antioxidant levels, aging and chronic illnesses as shown below;

FIG. 2: ANTIOXIDANT BLOOD LEVELS AS A MARKER



The drop in blood antioxidant levels coincides with the decrease in the absorption of vitamins and minerals with corresponding decrease in the production of antioxidant enzymes in the body and visible signs of aging begin to show up at 40 years. The antioxidant defense system becomes less efficient. About 40% of men may have erectile dysfunction usually associated with some degenerative condition at 40 years of age.

At 50 years, there is sharp decline in the blood antioxidant level within 10 years which coincides with a 69 – 70% incidence of erectile dysfunction with more of them associated with diabetes, hypertension or arthritis, etc. With advancing age and decreasing blood antioxidant levels, the repair mechanism at the cellular level is slow and cellular function is less efficient in an environment of excess free radicals. Chronic illnesses progress faster in such states.

After 50 years, organ size may actually shrink in most people due to loss of cells by death. As the glutathione, an antioxidant enzyme that is critical in metabolic activity in mitochondria drops, lethargy sets in, followed by getting tired easily. As glutathione levels drop below 80% in cells, cell deaths begin to occur with few or no new cells being produced in the organ. Organs shrink in size and organ efficiency begins to decline. The average life span, at 72 years, coincides with a very low antioxidant levels in the blood. About 70 – 90 illnesses and disease states are currently linked to or associated with oxidative stress and free radical injury. More will be added to this list.

Sepsis is a range of clinical conditions observed when the body is invaded by pathogenic microorganisms or when it suffers an infection by bacteria or viruses. Their toxins generate free radical chain reactions in the body and it experiences a generalized or localized oxidative stress. So, sepsis may not be the difficult-to-treat syndrome it is made out to be, if research shows that it is nothing more than an oxidative stress limited to a part of the body or an organ such as the heart or liver or lungs or generalized in the whole body.

Previous use of antibiotics, injuries and wounds, alcohol and drug abuse or infections all have two things in common – they are risk factors for sepsis and they deplete antioxidants in the body. Vitamin C levels drop in lung cells or in T4 cells depending on the source of infection. Under severe and prolonged oxidative stress (especially in malnourished people), inflammations may result and the infection may spread easily to organs in the body. The usual low body temperature may be a sign of oxidative stress on the Krebs cycle, low metabolic activity in cells and low energy production accompanied by muscular pains or general weakness. It may be characterized by low glutathione levels and low vitamin levels. Clinical nutrition may become the first choice in such cases along with the need to assess the infection or other symptom and treat it.

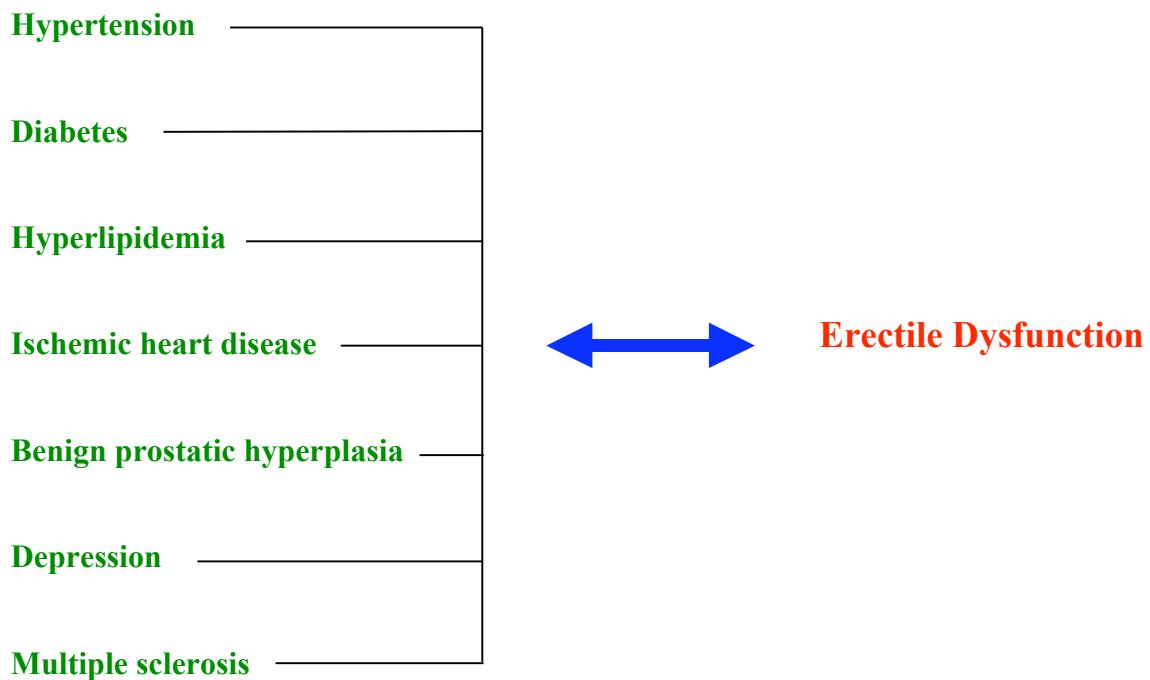
Severe depletion of vitamin C in the body at levels below 40% might create a sepsis condition and if glutathione levels also fall 30% the condition may appear critical. If vitamin and glutathione levels become further depleted by free radical generating drugs, the chances of recovery may be poor as in the case of 20% of such cases. Research in this direction will yield practical insights and might alter the course of treatment.

Cancer cells are created through a transformation process. One transformation (Type I transformation) process involves the shutdown of the Krebs cycle through severe oxidative stress on intermediates and enzymes which become inactivated. The cell then resorts to anaerobic respiration for energy through a process (of fermentation) that generates a host of free radicals that help in the proliferation of cancer cells and tumor

formation. The other transformation (Type II transformation) takes place through excessive hydroxyl radicals that attack genetic material and cause breaks in the DNA. Further research may reveal the gene splicing activity of such highly reactive free radicals and repair of the DNA in excess free radicals may result in aberrations. Exposure to chemicals that have free radical generating toxicity may have a similar effect. Vitamin E in the testes may be a protecting factor for minimizing such free radical injury during spermatogenesis. Type I transformation may be reversible but Type II transformation may not be reversible and these cancer cells may have to be destroyed by naturally occurring cytotoxic compounds found in certain herbs and spices.

Adequate levels of antioxidants in the body can prevent both types of cell transformations or lower the risk of oxidative injury as shown by the many studies on the incidence of cancers in people with a high or low intake of antioxidants or vitamins. Besides, there are a host of chronic conditions that seem to occur in clusters or progress into a cluster of problems that may include erectile dysfunction (ED).

FIG. 3: ERECTILE DYSFUNCTION AND FREE RADICALS



Erectile dysfunction (ED) generally occurs in older men affecting about 40% of men at around 40 years and increasing to 69 – 70% at around 50 years. The first observation is that it coincides with decreasing levels of antioxidants in the blood and is more common among smokers. Cigarette smoke contains more than 300 toxic chemicals that generate free radicals in the body, cause oxidative stress and deplete antioxidants.

Erectile dysfunction is usually found in association with other health problems such as hypertension, diabetes, arthritis, cardiovascular disease, etc. When ED develops in a person at an early age it serves as an indicator, in fact as an early warning sign for generalized degenerative diseases especially cardiovascular disease, diabetes and arthritis. ED is an effective diagnostic tool in men below 35 years because it is a marker for free radical damage that will affect other systems of the body over time. So, ED and its associated health problems have a two-way link as shown above.

Erectile dysfunction is a common sequel of diabetes mellitus. About 35 – 75% of diabetic men develop ED². Tarek and his team reported elevated levels of nitric oxide (synthase isoforms NOS) in diabetic penises compared to controls which returned to normal following insulin therapy showing the regulatory effect of insulin and the nitric oxide mediation in ED³.

A better way to depict the relationship between these clusters of conditions is as shown in Figure 4 below;

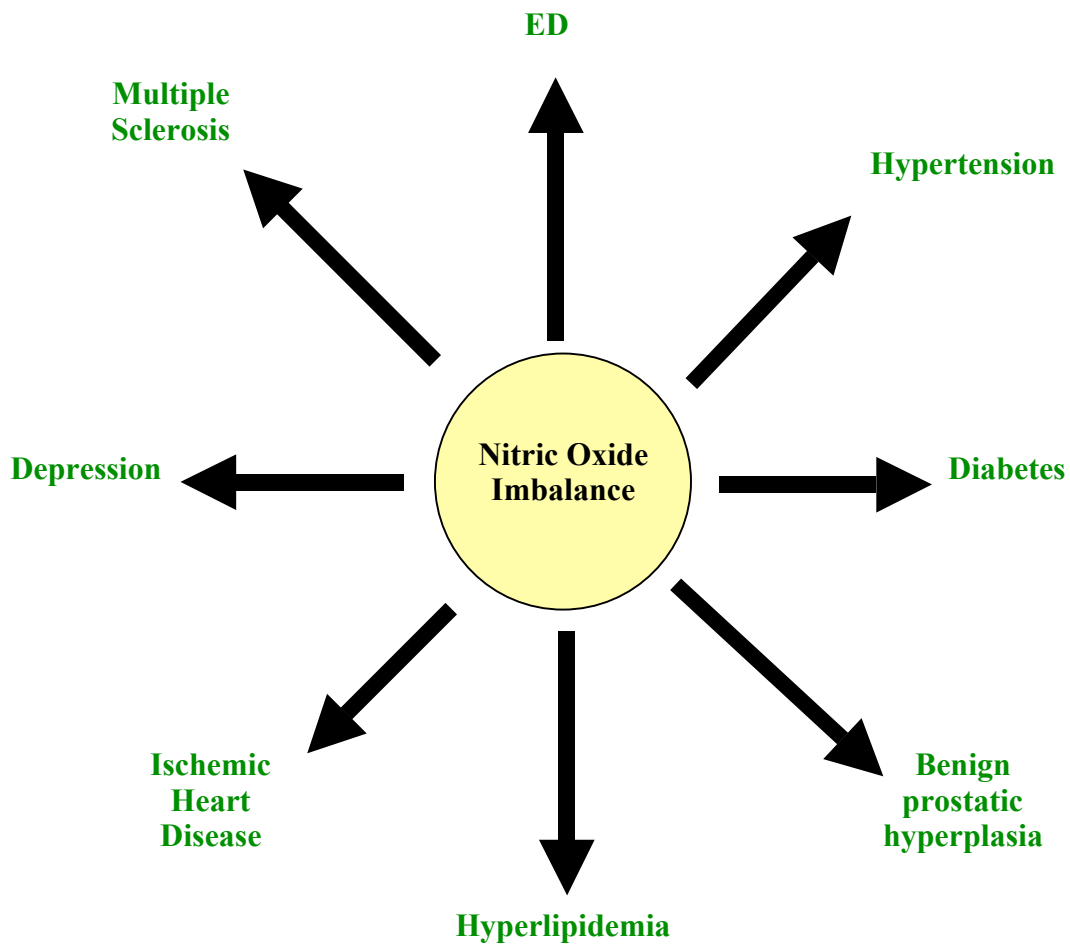


Figure 4: Nitric Oxide Imbalance Mediated Disease Conditions

“Penile erection depends on the balanced action between antagonist vasoactive molecules such as nitric oxide (NO) and angiotensin. Endothelial nitric oxide synthase (eNOS) and

angiotensin – converting enzyme (ACE) polymorphisms have been associated with endothelial dysfunction which is described as a cause of ED. Researchers at the Human Medical Genetics Hospital in Mexico show the very interesting fact that there are independent factors for ED including diabetes mellitus, hypertension, cardiac disease and cigarette smoking but the Massachusetts Male Aging Study showed a critical link between coronary artery disease, diabetes and ED⁴.

This link can be better understood if we consider the following; Aging is thought to alter nitric oxide levels⁵. Smoking leads to decreases in nitric oxide synthase activity in the penis because of its effects on the vascular endothelium⁶. The extent of coronary artery disease correlates with the prevalence of ED⁷ and cardiovascular risk factors similarly exert an adverse effect on coronary endothelial function⁸.

There appears to be a biochemical link originating in the healthy functioning of the coronary endothelium that shows a correlation between cigarette smoking, hypertension, cardiac disease, diabetes and ED so much so that a group of experts agree that “the critical association between ED and cardiovascular disease lies with the vascular endothelium”⁹ and many experts agree that ED may itself be an independent marker for coronary artery disease¹⁰.

The healthy functioning of coronary endothelium must be recognized as one of the keys to optimal health. It plays a vital role in the production of NO and to maintain its balance in the bloodstream. The NO pathway is of primary importance to overall health. Excess production of NO or its deficiency can cause problems.

Researchers have also shown the damaging effects of radical oxygen species leading to an oxidant/antioxidant imbalance with the NO(-) radicals associated with rheumatoid arthritis (RA) and the oxygen radicals being associated with osteoarthritis (OA)¹¹. Nitric oxide is the most important molecule in the mediation of chronic diseases and it also points the way to manage better health by considering coronary endothelial health.

Another study indicates that excessive NO caused by endothelial dysfunction is associated with excessive amounts of superoxide and peroxynitrate from superoxide and nitric oxide leaving reduced amounts of endogenous nitric oxide from endothelial cells for vasodilation¹² and a large number of radicals in the bloodstream that could cause oxidative stress in the pancreas or heart and reactive oxygen species that oxidize LDL. Oxidized LDL sticks to the walls of blood vessels in the heart and elsewhere and precipitate atherosclerosis or inflammations in the body.

Nitric oxide is transformed into peroxynitrate by the reactive oxygen radical and it degrades vitamins and glutathione and other protein and hormone molecules in the body. To protect vitamins and glutathione in the body, other biomolecules are needed, which may be called super-antioxidants because they donate electrons to free radicals and radicals more readily than vitamins and glutathione and prevent the latter from degradation, so that the vitamins and glutathione and other antioxidant enzymes may be used in the Krebs cycle and in mitochondrial metabolic activity. These super anti-oxidants are bioflavonoids and anthocyanins.

The nitric oxide balance as managed by a healthy coronary endothelium is the key to maintaining optimum health. Excess nitric oxide promotes disease through free radical generation such as arthritis, cardiovascular disease, diabetes, etc. Overproduction of the

NO (reactive) free radical causes tissue damage and chronic inflammation pathology. Duke University Medical Center researchers confirmed that nitric oxide plays a major role in assembling protein networks that direct fundamental cell activities such as whether cells live or die¹³ which is a much broader role than suggested earlier such as delivery of oxygen to tissues. Cell death is linked to a number of health problems including heart failure. Nitric oxide is now recognized as playing an important role in the regulation of diverse physiological processes¹⁴.

Impaired NO production is equally bad and results in disease. There are studies to show that nitric oxide deficiency occurs in end stage renal disease (ESRD)¹⁵. In systemic sclerosis patients, the endothelial cells fail to produce nitric oxide as evidence from low levels of nitric oxide in cultured peripheral blood monocyte/macrophage cells¹⁶. Infants suffering from persistent pulmonary hypertension (PPHN) had lower levels of citrulline, arginine and nitric oxide metabolites¹⁷.

Impaired ability to generate nitric oxide is also now suspected to cause diabetic neuropathy¹⁸. Individuals with Duchenne muscular dystrophy (DMD) also have low levels of nitric oxide as seen from the low levels of the enzyme nitric oxide synthase (NOS). Type 1 diabetics also have low levels of nitric oxide in their system.

Martin¹⁹ of Washington State University has cited studies showing excessive nitric oxide in fibromyalgia (FM). Excessive nitric oxide leads to the generation of free radicals than generate pain and in traumatic head injury, the body may be generating excess free radicals to protect the brain from infection. Nitric oxide generated by T4 cells is part of the body's defense mechanism. When these cells surround a pathogen or a parasite they release concentrations of free radicals and the highly diffusible nitric oxide radical readily enter these microorganisms and disrupt their metabolism and cause their death while the T4 cells resist oxidative injury by high concentrations of vitamin C in them. Children with low levels of nitric oxide will naturally suffer severe symptoms of malaria due to the slower rate of killing of the parasite. Hence, herbs that stimulate free radical activity in the body have been successfully used by Chinese physicians to treat malaria.

Elevated homocysteine levels in the blood are associated with incidence of heart attacks or strokes. Homocysteine may interfere with the functioning of the endothelium. In certain conditions it may trigger the endothelium to produce excessive nitric oxide. Native LDL increases the production of nitric oxide and superoxide which increases the likelihood of the formation of the reactive peroxynitrite radical (ONOO-),²⁰. The peroxynitrite radical exhibits a wide range of oxidative cytotoxic properties²¹ including in plague formation²².

The level of NO production by the endothelium is an indicator of health problems and disease states or even cell death. Early in the disease state or prior to it there will be an imbalance in the oxidative/antioxidative enzyme system. In one situation, there may be an overproduction of NO and superoxides leading to an excess of the reactive peroxynitrite radicals producing a wide range of disease conditions. In another situation, the enzymes in the Krebs cycle in the endothelial cells and the metabolic pathways come under oxidative suppression and are inactivated showing lower levels of citrulline, arginine and NO production which may not improve by increasing arginine. Different disease conditions may result from excessive NO levels and low NO levels. Metabolic enzyme inactivation may also be the cause of low levels of nitric oxide synthase (NOS).

Low levels of nitric oxide in the fetus may be due to inactivation of metabolic enzymes by oxidative stress that may be associated with developmental defects that may be widespread or confined to organs depending at which stage of its development the oxidative stress occurred. In some rare cases the developmental defects may look like deformed development associated with chromosomal aberrations. The cells in the lungs are known to produce nitric oxide and oxidative stress in the lungs later during fetal development may result in low nitric oxide levels in these cells and produce structural defects.

Nitric oxide is critical to normal physiological processes associated with normal development and health and its excess or low levels will produce developmental defects or pathological problems. The multifaceted role of NO in the human body must be understood so that clinical nutrition as an intervention for restoring health can be effective and it must attempt and succeed to restore endothelial function as a way to address the ravaging effects of excess nitric oxide.

Macrophages in the bloodstream that are part of the immune system, produce excess nitric oxide and other cytotoxic radicals in order to kill bacteria that is engulfed within it. Nitric oxide also serves as a neurotransmitter between nerve cells. NO also plays a role in development and maintenance of erection by stimulating PDE5-related intracellular cGMP in the smooth muscle cells surrounding the blood vessels in the corpus cavernosum. The increasing levels of cGMP promotes relaxation of these muscles and more blood flows in the corpus cavernosum. Increasing levels of cGMP that result lead to vasodilation and hence erection. NO has useful biological role in healthy physiology, provided it is not produced in excess by the endothelium and not produced in diminished amounts in the tissues of organs.

However NO is a free radical and by nature it is very reactive and unstable. In the body, it reacts rapidly with ROS to form the poisonous nitrogen dioxide and other reactive species (see;Fuhua et al, Molecular Mechanisms of Increased Nitric Oxide (NO) in Asthma: Evidence for Transcriptional and Post-Translational Regulation of NO Synthesis, The Journal of Immunology, 2000, 164: 5970-5980). Reactive oxygen species (ROS) avidly reacts with nitric oxide (NO) producing cytotoxic reactive nitrogen species capable of nitrating proteins and damaging other molecules which can be ameliorated with antioxidant therapy. Amelioration of oxidative stress by high-dose vitamin E enhances NO availability, improves hypertension, lowers protein nitration products (Nosratala et al, Enhanced Nitric Oxide Inactivation and Protein Nitration by Reactive Oxygen Species in Renal Insufficiency, Hypertension. 2002;39:135).

There is also accumulating evidence indicates that reactive oxygen species (ROS) are associated with the different steps of carcinogenesis. Researchers have found increased NO• production and MDA levels in plasma of patients with gastric cancer. These increases can be associated with the oxidant–antioxidant status in these patients (Ebubekir et al, Nitric Oxide Levels and Lipid Peroxidation in Plasma of Patients with Gastric Cancer, Japanese Journal of Clinical Oncology 32:162-166 (2002). The association of excess NO with lipid peroxidation products in cancer patients is interesting and points to oxidative damage to the cell wall of cells, that in turn produces the charge observed in cancer cells. The charged cell membrane turns into a ROS generator as oxygen molecules pass through it and increase the ROS population in the cancer cells turning the cytoplasm acidic (see:Cancer Therapy - Fat Soluble Antioxidants or Chemo Drugs, La Leva di Archimede, 2006).

The presence of multiple areas of hypoxia (low oxygen tension) is a hallmark feature of human and experimental tumors (Claire and Craig, Macrophage Responses to Hypoxia - Implications for Tumor Progression and Anti-Cancer Therapies, American Journal of Pathology, 2005;167:627-635) but low oxygen characterized by relatively higher ROS in cartilaginous tissues at the joints may initiate biochemical pathways that lead to degenerative changes and wasting, instead.

NO and its products can stimulate and inhibit lipid peroxidation. The effects of $\cdot\text{NO}$ on lipid oxidation depend on relative concentrations of $\cdot\text{NO}$, reactive oxygen species, and antioxidants, with all interactions in turn influenced by the aqueous-lipid solubility and relative rates of reaction of the participating reactive species (Kissner et al, Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. Chem Res Toxicol. 1997;10:1285–1292. (Erratum. 1998;11:557.)) [Medline]:Hogg et al, Nitric oxide and lipid peroxidation. Biochim Biophys Acta. 1999;1411:378–384. [Medline]:Patel et al, Biological aspects of reactive nitrogen species. Biochim Biophys Acta. 1999;1411:385–400. [Medline] The influence of $\cdot\text{NO}$ on lipid oxidation depends on the relative concentrations of $\cdot\text{NO}$ and $\text{O}_2\cdot^-$ -and organic peroxy radicals ($\text{ROO}\cdot$) present in the immediate vicinity).

Not every one with excess NO will have the exactly the same disease states. It will depend on the tissues where the action of the NO and its products and the relative abundance of antioxidants in the tissues. It is clear from experimental studies that amount of lipid peroxidation will depend on the relative concentrations of NO and ROS and peroxy radicals in the immediate vicinity and the relative concentrations of ROS scavengers and fat soluble antioxidants.

NO stimulates lipid peroxidation because of ONOO⁻ formation (Rubbo et al, Nitric oxide inhibition of lipoxygenase-dependent liposome and low-density lipoprotein oxidation: termination of radical chain propagation reactions and formation of nitrogen-containing oxidized lipid derivatives. Arch Biochem Biophys, 1995;324:15–25 [Medline]).

NO reacts with ROS ($\text{O}_2\cdot^-$) to form the reactive peroxynitrite (ONOO⁻) that reacts within a second. with proteins, lipids, carbohydrates, and DNA of subcellular organelles and cell systems through oxidation and nitration mechanisms. ONOO⁻ also readily reacts with carbon dioxide (CO_2) to form a highly reactive nitrosoperoxocarbonate intermediate (ONOOCO²⁻), (Beckman et al, Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol. 1996;271:C1424–C1437.). So, even with excess NO and carbon dioxide in cells, there can arise health problems.

Since the disease states can be mediated through oxidative injury arising from oxidative damage to proteins, lipids and carbohydrates, the inflammations produce a wide range of conditions as observed in the many forms of arthritis, aptly referred to as arthritic disorder. In some forms, damage to proteins may include damage to proteins involved in the oxygen transport system or increase in pyruvate build-up but the degenerative changes are due to inhibition of collagen formation on account of depletion of vitamin C that is spent in ROS scavenging. Vitamin C is essential in collagen formation. Over time, the NO-ROS products may damage the healthy biochemical pathways in the cells at the joints and produce pathological states and pain. Damage to glucose molecules and protein molecules may precipitate glycation and the production of glycoproteins. Damage to insulin molecules could result in type II diabetes while excess ROS-NO reaction in the pancreas may reduce insulin production.

It is clear that the end results of excess NO in the causation of disease states depends on a number of factors present together and the concentrations of these factors may vary in different individuals, producing different results that may vary from chronic degenerative disease to cancers. And two of the key factors are natural vitamin C and fat soluble antioxidants. When the ROS-NO products are sufficient in concentrations, they can disrupt normal and healthy biochemical pathways, reduce protein output and even produce deformations in bones.

"The regulation of nonenzymatic and enzymatic lipid oxidation reactions by nitric oxide ($\cdot\text{NO}$) is potent and pervasive and reveals novel non-cGMP-dependent reactivities for this free radical inflammatory and signal transduction mediator. $\cdot\text{NO}$ and its metabolites stimulate and inhibit lipid peroxidation reactions, modulate enzymatically catalyzed lipid oxidation, complex with lipid-reactive metals, and alter proinflammatory gene expression. Through these mechanisms, $\cdot\text{NO}$ can regulate nonenzymatic lipid oxidation and the production of inflammatory and vasoactive eicosanoids by prostaglandin endoperoxide synthase and lipoxygenase. When endogenous tissue rates of oxidant production are accelerated or when tissue oxidant defenses become depleted, $\cdot\text{NO}$ gives rise to secondary oxidizing species that can increase membrane and lipoprotein lipid oxidation as well as foam cell formation in the vasculature, thus promoting proatherogenic effects. In summary, $\cdot\text{NO}$ is a multifaceted molecule capable of reacting via multiple pathways to modulate lipid oxidation reactions, thereby impacting on tissue inflammatory reactions (Allison et al, Nitric Oxide Regulation of Free Radical- and Enzyme-Mediated Lipid and Lipoprotein Oxidation, Arteriosclerosis, Thrombosis, and Vascular Biology. 2000;20:1707).

Clinical nutrition places an obvious perspective on antioxidants and vitamins and their role in maintaining the natural nitric oxide balance in the body, promoting the normal and healthy biochemical pathways for health as well as sexual health. They are vital to coronary endothelium health, nitric oxide balance and levels of hormones in the body. Vitamin A is used in the conversion of cholesterol into estrogen in the female body and androgens in males which indicates one of the important reasons for taking sufficient amounts of cholesterol. It is also used for building the lipid layers in cell walls. The drop in vitamin A may explain the decline in hormone levels with advancing age.

Vitamin A also recharges vitamin E which plays an important role in protecting cellular membranes from oxidative stress. Like omega-3 fish oil, vitamin E protects heart cells from oxidative stress and prevents arrhythmia and sudden heart failure.

In healthy individuals, vitamin E is found in abundance in ovaries and testes. In females, it protects the ovarian follicles from oxidative damage while in males it may work to prevent oxidative injury by the free radicals to the genetic material during spermatogenesis. But administering vitamin E alone may not provide the expected protective effect because it is recharged by vitamins A and C and excess vitamin E intake may be harmful as it depletes vitamin A and C and reduces their protective effect.

Excessive free radicals in the bloodstream can cause blood clots and oxidize LDL which then attaches to arterial walls and forms plaques. Vitamin C prevents the oxidization of LDL and prevents or minimizes the risk of cardiovascular disease. Nearly 50 years ago, a Canadian cardiologist, JC Patheson (MD) reported that more than 80 percent of his heart disease patients had low vitamin C blood levels. This is corroborated by a University of California study concluded in 1992 that increasing vitamin C intake nearly halves the death rate from heart disease and extends life expectancy by up to six years. Vitamin C

and E may be important antioxidants that prevent platelets from clumping together. These clumps can cause blocks in capillaries in the heart.

The water soluble vitamins are easily depleted by free radicals in cigarette smoke and explain the higher incidences of nitric oxide imbalance induced diseases, lower hormonal levels, higher incidences of cardiovascular disease, ED, heart attacks, strokes, diabetes and cancers in smokers.

When vitamin A is depleted an important factor that recharges vitamin E is lost and may explain the loss of libido as well as the ability to prevent oxidative damage to genetic material. The increase in the smoker population and the increasing amount of free radicals from automobiles over the last 30 years and the use of immunotoxic medication and drug abuse may be the most critical link to the 1% annual increase of cancer around the world.

Vitamin C is the body's frontline free radical scavenger whether in the eye lens, nervous tissue, lungs or in immune system and evolutionary biology chose the right biomolecule because it can be recharged by many other antioxidants. It should be taken at least twice a day because being water soluble, it is excreted through urination.

Vitamin C is depleted in conditions caused by or that produce free radicals such as inflammations, arthritis and infections. Vitamin C is easily depleted by the millions of free radicals generated by cigarette smoke and it begins to have a depleting effect on the alpha-lipoic antioxidant network and finally affects cellular functions and the ability of T4 cells to protect itself from the free radicals they generate to destroy pathogenic bacteria.

The Industrial Revolution was all about machines and their role in economies. This revolution had its impact on health after WWII by increasing air-borne pollutants while industrial scientists were busy developing and introducing toxic chemicals into the environment. These pollutants, industrial chemicals and drugs added tremendously to the arsenal that generates free radicals in the body.

There were other negative effects. Take rice milling, for instance. Thiamine and B vitamins are essential for carbohydrate metabolism as their coenzymes are used in several reactions to breakdown glucose to release energy in aerobic respiration. A block in this energy production coupled by oxidative stress on the Krebs cycle and its enzymes may promote cancer cell transformation. Also B vitamins along with vitamin C prevent inflammations.

Nature had already coated each grain of rice with B vitamins but the industrial revolution took away this precious antioxidant resource that came with the carbohydrate by a milling process and we end up eating polished rice. Is it any wonder that the incidence of inflammations is also on the rise and is found also in children?

When children with skin rashes or inflammations of the skin are brought to a medical practitioner, they either get a vitamin supplement or steroids depending on the training received by the medical practitioner.

Another perspective on health and aging is the "very interesting feature of the loss of reserve due to the decreasing number of cells in each organ. Since free radicals kill cells, the cumulative effect of trillions of reactions results in the loss of its reserve. Muscle strength decreases, lung capacity decreases, filtering capacity of the kidneys decrease and

so on".²³ In later years, the number of new neuronal cells formed in the brain decreases and it begins to shrink. Naturally, when organs lose all the reserve cells, their functioning capacity is set to decline. When it falls below the level required for optimal health, there is a visible decline in health status.

When selenium intake falls along with the levels of antioxidants in the blood, the production of selenium based antioxidants also reduces, thereby producing a decline in mitochondrial metabolic activity. Similarly, prolonged oxidative stress also depletes the selenium based antioxidant network with disturbances in the functioning of the endogenous-exogenous antioxidant network and severe depletions disturb cellular function and can cause cell death resulting in the loss of the reserve.

When one recognizes the critical importance of the decline in the antioxidant system of the body and its impact on cells and organs, it becomes easier to develop approaches in clinical nutrition to arrest the damaging effects of excess free radicals in the body on cell membranes, LDL, biomolecules produced by the body and optimal cellular function. Some of those biomolecules such as melatonin produced by the brain during sleep are extremely important because melatonin is a powerful brain-body antioxidant and more of it may be produced in persons without sleep disturbances and in a brain that are not under any oxidative stress. By maintaining antioxidant levels close to "peak levels" may prevent or delay reserve cell loss and shrinking of muscles and organs.

There is no question about the role of vitamins or the antioxidant network or the levels of antioxidants in the blood in the healthy functioning of the Krebs cycle and optimal cellular function which translate to youthful health or optimal health but better research studies must be designed to show their clinical efficacy and in therapeutic use rather than the simple link between a vitamin with a specific deficiency.

Herbs have been used for medicinal purposes and considerable research shows the beneficial effects including, anti-tumor or cytotoxic activity or antiplatelet properties or anti-inflammatory activity and their use to reduce the risk of cardiovascular disease or cancers. The research on the role of antioxidants in preventing LDL oxidation and the role of black pepper in reducing LDL in the blood is impressive but clinical nutrition has not yet designed approaches for therapeutic effect such as using vitamin C and E and black pepper in conjunction as an approach in patients with cardiovascular disease and how it prevents cardiovascular disease in healthy individuals.

The ratio of HDL/LDL is an important ratio when considered along with the amount of triglycerides. However, low levels of LDL alone may not be a good indicator of low risk of cardiovascular disease in the future. It must be considered against blood antioxidant levels. Excess free radicals, as in the case of smokers or low blood antioxidant levels in older people, above the 60 age group, not only increases the prospects of blood clots but also increases the risk of LDL oxidation. Oxidized LDL poses the real risk as it sticks to walls of arteries and initiates plaque formation. Hence, people with high HDL/LDL ratio and a high daily intake of antioxidants along with black pepper should have the lowest risk as a group.

Death, it seems certainly comes in old age with the depletion of blood antioxidant levels and the resultant oxidative stress in the heart or kidneys or nervous tissue and disease states in the various tissues and organs are similarly produced by excess free radicals that exert oxidative stress on the Krebs cycle or the genetic material in cells. Before the advent of allopathic medicine, our ancestors depended on herbs for antioxidants to address

disease conditions and today researchers are shedding more light on the antioxidant properties of herbs and their use in restoring health or in certain diseases. The research on some herbs is tabulated below.

Table 2: Antioxidants In Herbs In Anticancer Activity

Herb	Antioxidant Activity
Garlic	Antioxidant activity in lymphocytes and macrophages enhances their natural role in destroying cancer cells. Lowers risk of cancer in distal colon in women by 50%. ²⁴ Effective in small tumors and slows down cancer proliferation in bladder, stomach, colon and skin. ²⁵
Onion	High consumption of onion and other Allium species (garlic) reduces incidence of stomach cancer and lowers mortality rates from stomach cancer by 50% ²⁶ due to its antioxidant activity in cells of the stomach.
Flaxseed	The lignans in flaxseed are converted into mammalian lignans (enterolactone and enterodiol) by bacterial fermentation in the colon ²⁷ and the antioxidant activity of these lignans protects the selenium based antioxidant enzyme system involved in mitochondrial metabolic activity. These lignans may be more effective in slowing down metabolic activity in breast cancer cells and help to slow down their proliferation.
Turmeric	The antioxidant activity of curcumin (a diferuloylmethane) suppresses the development of cancers in the stomach, breast, lung and skin. ²⁸ Ginger rhizome contains curcumin together with a dozen phenolic compounds known as gingerols and diarylheptanoids. The antioxidant activity of these compounds is higher than that of tocopherols ²⁹ and effectively prevents cancer formation due to the additional role of selenium in ginger. Selenium is needed to produce the selenium based anti-oxidant enzymes.
Leaf Herbs, rose petals, saffron, annatto, carrots, palm oil	Carotenoids and anthocyanins are coloring pigments. They are very effective antioxidants in the primate body as they donate their electrons more readily than vitamins to neutralize free radicals and protect the Krebs cycle from oxidative stress and prevent the transformation of normal cells to cancer cells. ³⁰ They prevent immune suppression and protect the endocrine system from oxidative stress. Anthocyanins may also protect cell walls from free radical injury. Persons with high serum concentrations of carotenoids have a reduced risk of both cardiovascular disease and cancer.
Green Tea	Green teas contain polyphenolics, flavonoids and other phytochemicals which prevent cancer formation and cancer proliferation by inhibiting metabolic activity that synthesizes DNA in leukemia and liver tumor cells. ³¹ Phytochemicals in green and black tea may have antioxidant activity in thymus T4 cells.

Source: Winston JC, AM Journal Clinical Nutrition, Vol. 70 No. 3, 491S-499S Sept 1999.

In clinical nutrition, the importance of biological rust called lipofuscin will grow over time as a sign of free radical damage and an unhealthy state. Lipofuscin is an aggregation of molecules comprising damaged cell components (or cellular debris) and oxidized fatty molecules that have been broken up or reduced. It can be seen on sun damaged skin on the back of wrists as brown spots. This same biological rust may be deposited on or within the various organs of the body including the heart, brain, eyes etc and represents a sign of free radical success action.

Between life and death is the story of health as played out by free radicals and antioxidants. It should clearly come across to the mind that only an antioxidant revolution will help restore public health and reduce the cost of treating diseases in hospitals. There is a greater need for natural antioxidants today because very little can be done to reduce the free radicals in our environment and to reduce toxic chemicals used in industry as they are mostly related to jobs and economy. It is very difficult to get governments and nations to take a concerted and serious view of the situation and its impact on health but we need actually clinical research rather than mere information about anticancer properties or health benefits.

There are other problems as well. Science literacy is not very high among politicians. It is not an easy task to get them to see a holistic picture, such as human breast milk was designed by nature to be anticancer because cells are proliferating rapidly in babies and to confer some form of immunity as well, but human breast milk is today contaminated with cancer causing or cancer promoting pollutants putting the infant generation at risk. We have created a biologically strange world.

Then, there is the problem of big business and their lobbyist in state capitals who do a successful job for their pay masters. What they will tell politicians is that at Shenyang zoo, the tigers are more prone to disease compared to their wild ancestors due to pollution and due to eating food with additives – half of the old tigers are dying of cancers. It is the same story for the whole of mankind – low antioxidant levels in blood in disease states and in old age and additives in food that are either cancer promoting or generate free radicals in the body.

But clinical nutrition has no lobbyist at work although it may be taught at the world's top universities. So, although vitamin C is as potent as the heart drug, probucol which works as an antioxidant, doctors will prescribe probucol, the synthetic antioxidant because it is "pharmaceutically prescribed" and throw away millions of years of evolutionary biology that selected vitamin C as a universal free radical scavenger in the mammalian physiology and integrated it into the antioxidant network in the Krebs cycle.

Nevertheless, the importance of antioxidants and daily intake of micronutrients will grow in importance as a measure to prevent disease states, slow down the aging process and in clinical nutrition as ways are found to improve their administration in patients.

References

- ¹ Cutlet RG (MD), Am J of Clinical Nutrition: cf Antioxidants, OAK Publications, 2005, p.10
- ² Tarek M. et al., Lady Davis Institute for Medical Research, Quebec Urological Association
- ³ Haydee et al. J of Andrology, Vol. 25, No. 5 Sept/Oct 2004): cf www.aug.org/1997/23.html
- ⁴ Feldman et al., J Urol 1994, 151(1), 54 – 61.
- ⁵ Haas et al., Urology 1998, 51(3) 5/6 – 22.
- ⁶ Sullivan et al., BJU Int, 2002, 87(9), 838 – 45
- ⁷ Khan et al., Curr Med Res Opin, 2002, 18(1), 33 – 5
- ⁸ Khan et al., 2002 ca.
- ⁹ Jackson et al. 2002, ca; cf Postgraduate Medicine Online, Luciano Kolodny, Vol. 114, No. 4, Oct. 2003
- ¹⁰ Luciano ca.
- ¹¹ Veronica Rose, 2001, DGReview, Clinical Science.
- ¹² Yki-Jarvinen H et al., Annals of Rheumatic Diseases, 2003, 62:630 – 634; ARD online: Ischiropoulos H et al., Arch Biochem Biophys; 1992, 298:446 – 51 Medline
- ¹³ HealthDay, Feb. 2005
- ¹⁴ Barry et al., Toxicological Sciences, 59, 5 – 16, 2001
- ¹⁵ Rebecca et al., A J Physical Renal Physiol 276: F794 – F797, 1999, Vol. 276, Issue 5
- ¹⁶ Allanore et al., British Journal of Rheumatology, 2001, 40:1089 – 1096
- ¹⁷ Marshall Summar, The Reporter, 2001
- ¹⁸ Kevin and Yuen, Medical News Archive, WebMDHealth, 2002
- ¹⁹ ImmuneSupport.com, 2003
- ²⁰ Kirkwood et al., Circulation Research, 1995, 77:510 – 518
- ²¹ Koppenol et al., Chem Res Toxicol, 1992, 5:834 – 42
- ²² White CR, Proc Natl Acad Sci USA, 1994, 91:1044 – 48
- ²³ Bruce Miller, Antioxidants, OAR Pub., 2005, p10-11

- ²⁴ Steinmetz KA, Kushi LH, Bostick RM, Folsom AR, Potter JD, vegetable, fruit and colon cancer in Iowa women's health study; *Am J Epidemiol* (1994) 139:1 – 15.
- ²⁵ Dausch JG, Nixon DW, Garlic, A review of its relationship to malignant disease; *Prev Med* (1990) 19, 346 – 61, (Medline): Belman S, Onion and garlic oils inhibit tumor promotion; *Carcinogenesis*, (1983), 4, 1063 – 5 (Medline): Lau BHS et al., superiority of intralesional immuno-therapy with *Corynebacterium parvum* and *Allium sativum* in control of murine transitional cell carcinoma, *J. Urol* (1986), 136, 701 – 5 (Medline): Lau BHS et al., *Allium sativum* (garlic) and cancer prevention, *Nutr Res* (1990), 10, 937 – 48.
- ²⁶ You WC et al., *Allium* vegetables and reduced risk of stomach cancer, *J Natl Cancer Inst.* (1989), 81, 162 – 4, Dorant E et al., Consumption of onions and a reduced risk of stomach carcinoma, *gastroenterology* (1996), 110, 12 – 20 (Medline).
- ²⁷ Thompson LU et al., Mammalian lignan production from various foods, *Nutr Cancer* (1991), 16, 43 – 52 (Medline).
- ²⁸ Nagabhushan M and Bhide SV, Curcumin as an inhibitor of cancer, *J Am Coll Nutr* (1992), 11, 192 – 8: Chan MM and Fong D, Anti-inflammatory and cancer-preventive immunomodulation through diet – effects of curcumin on T-lymphocytes cf Huang MT et al., Food phytochemicals for cancer prevention, *Am Chemical Society* (1994), 222 – 30
- ²⁹ Kikuzaki H and Nakatani N, Antioxidant effects of some constituents, *J Food Sci* (1993) 58, 1407 – 10.
- ³⁰ Kohlmeier L and Hastings SB, Epidemiologic evidence of a role of carotenoids in cardiovascular disease prevention, *Am J Clin Nutr* (1995) 62 (suppl) 1370S – 6S: van Poppel G and Goldbohm RA, Epidemiologic evidence for beta-carotene and cancer prevention, *Am J Clin Nutr* (1995) 62 (suppl) 1393S – 402S: Morris DL et al., Serum carotenoids and coronary heart disease, The Lipid Research Clinics Coronary Primary Prevention and Follow-up Study, *JAMA* (1994), 272, 1439 – 41.
- ³¹ Lea MA et al., Inhibitory effects of tea extracts and (-) epigallocatechin gallate on DNA synthesis and proliferation of hepatoma and crythroleukemia cells, *Cancer Lett* (1993), 68, 231 – 6 (Medline).