EVOLVED REDUCED UTILIZATION OF SELENOPROTEINS IN HUMAN BIOCHEMISTRY?

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Two researchers claim that seleno-proteins evolved during the course of evolution and there is an "evolved reduced utilization of selenium-containing proteins" and this evolved reduced utilization opens the question of selenium suplementation. The two US-based researchers - Alexey Lobanov and Vadim Gladyshev of the University of Nebraska-Lincoln and Dolph Hatfield of the National Institutes of Health - are quoted below:-

> *"Selenium-containing proteins evolved in prehistoric times. Several human disorders have been associated with a deficiency in the trace element, among them are Keshan disease, a heart disorder affecting primarily children in certain provinces of China where the soil is deficient in selenium, and Myxedermatous Endemic Cretinism, a rare form of mongolism attributed to deficiencies in selenium and iodine found in certain areas of Africa. Selenium supplementation was thought to be necessary to prevent these and other diseases even in the areas with adequate selenium supply. The* ***evolved reduced reliance on selenium invites questions*** *regarding the widely accepted use of supplements incorporating this trace element to maximize amounts of proteins that rely on it. Supplements are taken without knowing which groups of the population can benefit.*
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> Gladyshev concludes:
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> *"The* ***evolved reduced utilization of selenium-containing proteins*** *in mammals raises important questions in human and animal nutrition. Selenoprotein expression is regulated such that people don't need to rely so heavily on dietary selenium which is* ***often present in excess amounts in the diet.*** *Individuals should consider their age, sex and medical needs before taking such supplements on a regular basis"(see: Health Freedom, Sepp, April 7, 2008,* Researchers call Selenium 'useless' mineral).

The four key issues in the above text are:-

1. Evolved reduced utilization of selenoproteins which is attributed to evolved reduced reliance on selenoproteins,
2. Medical needs or basic biochemical need in healthy biochemistry,
3. Groups that can benefit from supplementation or selenium and glutathione therapy, and
4. Heavy reliance on dietary selenium that is often present in excess.

The researchers correctly mention that human disorders are associated with selenium deficiency, among them is a heart disease called Keshan's disease. Technically, selenium deficiency is not the direct cause. Keshan's disease is a cardiomyopathy that affects young women and children in a selenium deficient region of China. It is associated with very low dietary intakes of selenium. Selenium supplements has been found to protect people from developing Keshan's disease but cannot reverse heart muscle damage once it occurs.

Glutathione (GSH) levels in human tissues normally range from 0.1 to 10 millimolar (mM), most concentrated in the liver (up to 10 mM) and in the spleen, kidney, lens, erythrocytes, and leukocytes[1]. In most cases, ordinary supplemental doses do not aid the body to reverse changes that are discernible and diagnosable, Usually, doses much higher than than the recommended daily allowance (RDA) are required. These doses must be obtained from food and edible substances and taken together with a cocktail of fruit juices mixed with a spoonful of medium chain fatty acids. Researchers are now turning to intravenous administration and topical sprays that are absorbed rapidly through the dermis to deliver the high doses for therapy as oral consumption of antioxidant degrades them in the tract. RDAs and normal amounts taken through the diet only serve to maintain the healthy biochemistry and is a strong preventive measure.

During normal cell metabolism, the superoxide called the oxygen free radical (OFR) is formed. When it is biochemically eliminated by its conversion into oxygen, hydrogen peroxide is formed. Reactive oxygen species (ROS) include oxygen ions, free radicals and peroxides.

In cardiomyocytes, a seleno-dependent form of GPx (Se-GPx) is the main enzyme responsible for the elimination of these reactive oxygen species (ROS)[2]. It is well established that in most industrialised countries, selenium intake is so low[3] and that it might limit GPx (gutathione) synthesis in the cells of the body[4]. The experimental work by Stephane and her co-researchers showed that selenium intake did not affect growth rate of the animals and the heart weight/body weight ratio, expressed as mg wet tissue per g body weight, remained similar in both groups. However, as expected, high selenium supply induced a significant increase in plasma (P-0.05) and RBC (P-0.01) selenium levels when compared to the Low-Se group. Moreover, total GPx activity was significantly increased in the myocardium of High-Se compared to Low-Se.

This study clearly demonstrates, that high selenium intake reduces the susceptibility of senescent rat hearts to ischaemia and reperfusion[5]. As for the observation that selenium status decreases with age in humans[6], Stephane et al, suggest that reinforcing selenium supply might improve the prognosis of cardiovascular diseases in old patients.

It has been shown for a long time that GPx (glutathione) functions as an antioxidant enzyme, reducing hydrogen peroxide and organic hydroperoxides[7] and **destruction** of mitochondrial

cristae and mitochondrial membranes and absence of mitochondrial glycogen have been associated with severe selenium deficiencies and mild deficiencies have also been proposed to be related to cardiovascular disease[8]. This represents the underlying biochemical cause of cardiovascular disease which is attributable to a reduced ability of the heart to eliminate hydrogen peroxide and organic hydroperoxides. Simply put, the whole of such research essentially means that the superoxide and hydrogen peroxide produced in cells must be eliminated by its conversion to water and oxygen, respectively, failing which the mitochondria suffer biochemical damage. Glutathione protects the cells from hydrogen peroxide toxicity. This biochemistry must be examined with some detail.

Understanding the basic underlying biochemistry reveals the actual initiation and causes of denegerative diseases such as cardiovascular disease or arthritis and diabetes. Without such understanding, one tends to state that obesity and cardiovascular disease (CVD) are causes of diabetes. That is obviously wrong because there are patients who have diabetes without CVD or arthritis and if no change in diet or antioxidant intervention occurs, many of them will develop CVD or arthritis in 6-8 years. Biochemical damage at the cellular and molecular levels caused by free radicals such as the reactive oxygen species (superoxide and the hydroxyl radicals) and highly reactive oxidants like the peroxynitrite that rob electrons from biomolecules in the body, when they are in excess. The resultant oxidative injury to these biomolecules and cell membranes leads to the development of disease conditions. Loss of integrity of cell membranes impairs their activity and cell output declines.

Free radicals, when in excess of the antioxidants in the cells, lead to oxidative stress. Diets and antioxidant therapeutic interventions function to remove oxidative stress by ensuring an excess of antioxidants. Antioxidants from food and edible substances in nano-form together with bioavailable minerals in ionic form can rapidly scavenge free radicals and remove the oxidative stress and promote the biochemical repair of biomolecules and cell membranes and reverse the degenerative changes by driving the healthy antioxidant-driven biochemical pathways in cells.

Glutathione is a tri-peptide thiol (sulfhydryl-containing) compound that can be administered intravenously. Whole fruit and vegetable (rich in glutathione, alpha-lipoic acid and coenzyme Q10 and foliate etc) extracts in nano-form can be sprayed topically and they are very quickly delivered through rapid absorption through the dermis and may be more effective than injectable glutathione as it might be degraded by the time it gets to the area of oxidative stress. Glutathione is a small molecule found in almost every cell. It cannot enter most cells directly and therefore must be made inside the cell. Hence, a much better approach is to provide all the ingredients in nano-form from food and edible substances that can readily enter cells to be reassembled quickly in them.

There are centres that now offer glutathione therapy where the therapeutic effects were sustained for two to four months suggesting that in untreated Parkinson Disease, glutathione has symptomatic efficacy. The solid part of the brain is largely cholesterol and lipids and glutathione prevents lipid peroxidation. Alpha-lipoic acid also prevent lipid peroxidation and it is a natural bio-chelater that binds to heavy metal ions and helps to remove heavy metal ions from the brain and body. Hence, alpha-lipoic acid must be used together with coenzyme Q10 and glutathione. Bioavailable mineral supplementation is of critical importance in such therapies.

In general, symptoms of glutathione deficiency may include generalized cell damage and depending where such damage has occurred, it can lead to symptoms of mental disorders, various nervous system disorders, tremors, and twitching or liver problems or kidney problems. Red cells are prone to burst, white blood cells decline in function and nerve tissue degenerates. A deficiency in glutathione associated with mitochondrial damage will lead to fatigue and later on to chronic fatigue and finally muscle wasting or even some forms of cancer.

Bioavaliable selenium, such as that found in food and edible substances like Brazil nuts, is itself an antioxidant and the biological system of plants and animals can route it into a biochemical pathway to produce glutathione which is a seleno-protein. Glutathione is a small protein comprising three amino acids – cysteine, glutamic acid and glycine. Selenium is required to produce glutathione and other seleno-proteins. It is a natural antioxidant enzyme that works within the body's antioxidant network system. That means, when it donates its electron to quench a free radical, the spent glutathione can be recharged by natural vitamin C or alpha-lipoic acid or it can be broken down to produce other useful biomolecules in the cells. It functions in the mitochondria to prevent oxidative damage caused by excess hydrogen peroxide and can prevent oxidative injury to the genetic material in the mitochondria called mDNA.

Glutathione works together with another antioxidant enzyme called catalase to convert hydrogen peroxide into water and oxygen. Hydrogen peroxide is moderately toxic to cell membranes and it is converted into life-giving molecules by the action of the natural antioxidant enzymes (glutathione and catalase) in the cells. However, excess superoxide (oxygen free radical – OFR) can react with hydrogen peroxide to yield hydroxyl radicals (OH-). Hydroxyl radicals are, therefore secondary radicals, and are highly reactive and can rob electrons from molecules near it or from molecules in the cell membranes and can cause oxidative injury in this manner to genetic molecules as well.

Oxidative injury within the mitochondria can decline the functioning of the cytochrome system and lead to a decline in the production of ATP – the energy molecule of cells leading to lowering of cell output and symptoms like fatigue. Oxidative injury to mDNA can lead to depletion of mitochondria in cells leading to chronic fatigue muscle wasting and the development of cancers.

The glutathione-catalase enzyme pathway protects the mitocondria and cells from potential oxidative stress and protects the healthy (antioxidant-driven) biochemical pathways by preventing the formation of the secondary radical (OH-) by converting hydrogen peroxide into water and oxygen:-

$$\text{Glutathione-catalase} + 2H_2O_2 \rightarrow H_2O + O_2$$

This reaction is more efficient when it proceeds catalytically in the presence of minerals like iron, copper, zinc and managanese. The depletion of these minerals by the excessive loss of MT proteins during chemotherapy and antibiotic administration compromises the functioning of this antioxidant pathway. **But, how is hydrogen peroxide produced in cells?**

During normal metabolism in cells, the superoxide (O_2^-) is produced. It is a free radical that will rob electrons from biomolecules in the cells. Such, oxidatively injured biomolecules cannot enter the healthy biochemical pathways. Oxidatively injured hormone molecules such as insulin will not be able to dock at their receptor sites and the glucose molecules cannot pass across the cell membrane into the cell. Oxidatively injured biomolecules can initiate free radical chain reaction that can be very detrimental to healthy biochemistry. Oxidatively injured glucose and protein molecules will react to form glycated proteins. Oxidatively injured lipo-proteins and LDL and vLDL will initiate plaque formation in the arterial walls. Excess superoxide can lead to excessive robbing of electrons from molecules in cell membranes leading to increase in cell membrane potential and inflammations.

Coenzyme Q10 is essential in another pathway that releases energy from ATP and oxidatively depleted coenzyme Q10 will effectively reduce the utilization of ATP in another pathway that releases energy for use in cellular biochemistry and effectively reduce cell membrane repair. Oxidative stress reduces the cell capacity to produce perforin and antibodies.

Excess superoxide also increases acidity in the cytoplasm. Increases in the pH will inactivate enzymes involved in the Krebs cycle. Consequently, if the Krebs cycle shuts down, aerobic energy is not produced and the altered cell biochemistry may reroute the glucose molecule into an alcohol pathway to meet its energy requirement, (through anaerobic respiration) effectively transforming it into a cancer cell. The superoxide is highly reactive and must be readily and quickly scavenged. An antioxidant enzyme called sodium dismutase (SOD) is required to play this role. But there is a catch. It does not convert the superoxide directly into oxygen. In biological systems, one of its main reactions is with itself (dismutation). The superoxide anion radical (O_2^-) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly and protects the cell from superoxide toxicity. This reaction can be represented as follows:-

SOD + OFR (O2.) --------> H2O2 + O2

That explains the production of hydrogen peroxide in cells and the inherently important role of the glutathione-catalase pathway to convert it into water and oxygen and prevent hydroxyl toxicity arising from the reaction of excess superoxide with excess hydrogen peroxide. And thus sufficient dietary intake of selenium becomes an issue in health.

Many drugs and chemicals yield high amounts of superoxide when they enter cells and excess use of drugs or their long term use can deplete SOD. At the same time, the depletion of minerals in the body will compromise the efficient funtioning of the glutathione-catalase system leading to health problems or increase the risk of disease conditions such as arthritis, CVD, diabetes, cancers, etc. The depletion of SOD by drug use during pregnancy and quite possibly by certain pain-killers for use in headaches and migraines may account for the increased incidences of cardio-problems such as hole-in-heart in infants. Naturally, glutathione depletion and selenium deficiency will lead to insufficient glutathione for use in the detox biochemical pathway and increases the risk of development problems and genetic damage.

Streptozotocin (Streptozocin, STZ, Zanosar) is one of the older chemotherapy drugs that is given by intravenously to reduce tumors of the pancreas. It is used in medicine for treating certain cancers of the Islets of Langerhans. It is a naturally occurring chemical. It can also kill bacteria. Streptozotocin is derived from the soil microorganism *Streptomyces achromogenes* and has also been synthesized. Ironically, it is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. Side-effects include nausea and vomiting and kidney and liver damage. The synthesized form may be more toxic than the naturally occurring compound.

Toxic drugs can disrupt healthy biochemistry in many ways. Drugs like streptozotocin and possibly other drugs that block the formation of glucose in cells can interfere with facilitative glucose transporters (GLUT). These proteins facilitate the transport of glucose through biological membranes. When these proteins are oxidatively damaged they cannot bind to glucose molecules and there is a resultant decrease in glucose transport. Similarly, when their production is suppressed by excess ROS caused by drugs, glucose transport also declines. A sharp decrease in glucose transport can lead to apoptosis through glucose deprivation-induced ATP depletion and stimulation of the mitochondrial death pathway cascade caused by inhibition in the production of adequate levels of antioxidant enzymes including glutathione which is, in fact a glucose deprivation-induced oxidative stress. Intracellular glucose levels drop rapidly as glucose transporter expression decreases. As a result of these rapid changes, reduced glutathione levels are depleted and DNA fragmentation occurs[9].

Naturally, because such drugs indirectly cause oxidative damage to the genetic molecules or chromosomes including fragmentation of DNA, they are carcinogens in humans and have the potential to develop cancers. There is sufficient evidence of streptozotocin carcinogenicity in

experimental animals. "When administered by intraperitoneal injection, streptozotocin induced increased incidences of kidney and lung tumors in mice of both sexes, and produced uterine tumors in female mice. When administered by intraperitoneal injection, streptozotocin increased the incidences of kidney and pancreatic islet cell tumors in rats of both sexes, liver tumors in female rats, and peritoneal sarcomas in male rats. When administered by intraperitoneal injection, streptozotocin induced cholangiomas and hepatomas in hamsters. When administered as a single intravenous injection, streptozotocin induced kidney adenomas, adenocarcinomas, and sarcomas in rats of both sexes. Intravenous injection of streptozotocin induced hepatomas in hamsters."[10]

A very interesting research in France on the transient increase of glucose in (hypothalmic) cells increased reactive oxygen species in the mitochondria (mROS). The researchers demonstrated that transient glucose challenge in the (hypothalamic) cells has the ability to trigger an increased ROS production that can be reversed with antioxidant treatment. Antimycin, a complex III inhibitor, that blocks the electron transport chain (ETC) in mitochondria also led to mROS production which was an identical response to that induced by glucose alone[11]. Transient increases in glucose tend to increase the metabolism and results in excess superoxide as superoxides are a byproduct of metabolism and excess superoxide can accumulate in mitochondria when its free radical quenching and antioxidant system suffers an inhibition. Both produce the same effect.

Antimycin A, an active ingredient in a pesticide called Fintrol, functions by inhibiting the oxidation of ubiquinol in the electron transport chain, ultimately preventing the formation of ATP. Ubiquinol is the reduced form of coenzyme Q10. Antimycin A binds to the Qi site of Complex III (the enzyme cytochrome c oxidoreductase), in the cytochrome b subunit and inhibits this complex resulting in the accumulation of large quantities of the toxic superoxide (OFR). It blocks the electron transfer process, which prevents ubiquinol to quench the superoxide. The excess superoxide can oxidatively suppress the protonation in the cytochrome c system and lower ATP output and later on also oxidatively damage the mitocondrial membranes and mDNA as well. The only way to protect mitochondria and mitochondrial function is to ensure that:-

1. Firstly, there are no inhibitors or blockers in the biological system that inhibit the quenching of free radicals by the natural antioxidants or otherwise block the production of ATP molecules, and

2. Secondly, there are sufficient natural antioxidants to quench free radicals and prevent oxidative stress and oxidative injury to biomolecules and biomembranes. For this to happen such natural antioxidants may have to be introduced exogenously.

The use of antimycin in this French study proves the cytotoxicity of such antibiotics that are used in pesticides and as fish poison. This cytotoxicity is mediated trough the excess superoxide that can then react with the excess hydrogen peroxide to rapidly produce a large number of hydroxyl radicals. Its target is the mitocondria where the MT proteins are found. These proteins sequester a large number of minerals that enable the protein to bind to hydroxyl radicals for removal across membranes and out of the cell to pass into the urine. It explains the loss of minerals as a result of the use of antibiotics. Fortunately, there are natural biomolecules that can kill bacteria without causing oxidative stress in mammalian cells or are otherwise not toxic like antibiotics.

Inhaled or ingested cyanide as used as method of execution in US gas chambers, almost instantly starves the body of energy by inhibiting the enzymes in mitochondria that make ATP due to extremely rapid build up ROS due to free radical chain reactions causing very rapid oxidative damage. Antibiotics and drugs work more slowly while chemo-drugs are more cytotoxic than most antibiotics. Mitochondria are the 'power-plants' in cells and are sites of metabolism and any disruption to the metabolism of mitochondria or damage to their structure leads to a wide range of disease conditions. These disease conditions arising from mitochondrial disorders often present as neurological disorders or manifest as myopathy, diabetes, multiple endocrinopathy, or a variety of other systemic manifestations [12] including cancers due to point mutations in mDNA or fragmentation of DNA.

Oxidative impairment in mitochondria causing mitochondrial dysfunction and leading to mitochondrial diseases include schizophrenia, bipolar disorder, dementia, Alzheimer's disease, Parkinson's disease, epilepsy, stroke, cardiovascular disease, retinitis pigmentosa and diabetes mellitus[13]. The common underlying biochemical cause in these seemingly-unrelated conditions in medical science is oxidative stress due to the accumulation of reactive oxygen species (ROS) and the formation of highly reactive secondary free radicals and oxidants like the peroxynitrite (ONOO-) that cause oxidative injury to biomolecules and membranes and disrupt healthy biochemistry and membrane function. These oxidants then damage the mitochondrial DNA (mDNA) resulting in mitochondrial dysfunction and cell death[14]. Only natural anitoxidants can effectively prevent oxidative stress and oxidative injury by being present in excess in order to quickly scavenge the superoxide and prevent the formation of secondary radicals and the highly reactive oxidant (ONOO-). SOD, glutathione, catalase and coenzyme Q10 are important in this process.

Research at the Broad Institute at Harvard University and the Massachusetts Institute also suggests that drugs like statins may interfere with processes in the mitochondria and lower ATP output[15] producing strong decreases in cellular ATP. Statins are also known to interfere in the

pathway that leads to the formation of coenzyme Q10 and any strong decreases of this enzyme in heart cells can pose a cardio-risk. An analysis of the diabetes drug, Avandia, in the New England Journal of Medicine reveals that it increased the risk of heart attacks. The FDA will require tougher warnings on diabetes drugs such as Avandia and Actos to strengthen warnings about a condition in which the heart does not adequately pump blood[16].

Mitochondrial disorders can be acquired while under drug treatment. AZT treatment in AIDS patients has been shown to cause mDNA depletion which in turn causes myopathic changes that are reversible upon termination of treatment. Chemotherapy agents such as fosfamide have been reported to decrease mitochondrial function. For mitochondria to reproduce themselves, a specific enzyme called gamma-DNA-polymerase or "pol-gamma" is required[17]. There is a link between the cell's ability to maintain integrity of its mitochondrial genome and mitochondrial genetic diseases[18]. Cardiomyopathy was documented with increased left ventricle (LV) mass, ventricular expression of atrial natriuretic factor (ANF) mRNA, mitochondrial ultrastructural defects with mitochondrial damage and myocardial depletion of glutathione[19]. It is clear that oxidative damage to mitochondria and mitochondrial depletion leads to muscle wasting and cardiomyopathy but blocks in cellular energy associated with coenzyme Q10 may be the cause of heart enlargement.

Many drug medications have been found to interrupt pol gamma. Studies suggest that virtually all the nucleoside analog reverse transcriptase inhibitors (NRTIs) including AZT interrupt pol gamma to some extent. One study has already demonstrated that people given AZT had significant depletion of mitochondrial DNA in muscle tissue. "Prior to HAART, long-term use of high-dosage AZT caused myopathy, cardiomyopathy, and hepatotoxicity, associated with mitochondrial DNA depletion. AZT is a known inhibitor of the mitochondrial pol-gamma and has been targeted as the source of the mitochondrial DNA depletion"[20]. So, free radical damage to mitochondria, whether by benzene and its derivatives or AZT or other toxic chemicals and drugs can cause the "chronic fatigue" and weight loss symptoms diagnosed in early AIDS patients. mDNA depletion can also be acquired from administration of NRTIs for AIDS[21].

SOD-1 catalyzes the conversion of superoxide radicals (O*2) into H2O2[22] and this role prevents oxidative stress and lipid peroxidation in the brain. Oxidative stress in the brain constitutes a major cause of neurodegeneration in the brain. Reactive oxygen species enhance lipid peroxidation and retard the ability of cells to handle calcium loads, leading to apoptotic cell death[23]. Oxidative stress is also a key component in the development and progression of diabetes complications, including diabetic nephropathy[24].

The higher incidence of leukaemia and malignant neoplasia of the breast, ovaries and rectum among persons over 55 years is a reflection of greater lipid peroxidation[25] as antioxidant levels

decline with age. They decline sharply after the age of 50. In women this period coincides with a decline in oestrogen levels. Oestrogen is a powerful antioxidant. With sleep disturbances melatonin yields also decline. Melatonin is a brain-body antioxidant that can be recharged by natural vitamin C and it works synergistically with this natural antioxidant.

Free radical reactions involving circulating lipids in the arterial wall and peroxides that induce endothelial cell injury produce changes in the arterial walls leading to the development of atherosclerosis[26]. Vitamin E treatment was demonstrated to significantly reduce the risk of cardiovascular deaths as well as non-fatal myocardial infarctions[27]. Vitamin E prevents lipid peroxidation and prevents platelet aggregation as well. Numerous studies have reported the protective effect of fruit and vegetable consumption on the incidence of disease and cancer[28]. Fat-soluble antioxidants like vitamin E prevent or slow down the rate of lipid peroxidation[29] Enzyme treatments are more effective in therapy when fat-soluble antioxidants are given together with water-soluble antioxidants while ensuring there are more than sufficient bioavailable minerals to enhance the catalytic function of these natural enzymes to:-

1. scavenge free radicals as quickly as possible,

2. ensure that free radicals are not in excess,

3. shut down alternative biochemical pathways established as a result of excess free radicals (oxidative stress), and

4. ensure excess natural antioxidants to drive biochemical pathways that are integral to health and yield useful biomolecules such as ATP, coenzyme Q10, seleno-proteins, hormones, repair proteins etc and yield cellular energy.

Drugs work in just the opposite direction because they generate the superoxide in the mammalian biological system. In many cases bioavailable selenium supplementation with fruit and vegetable juices is necessary to achieve therapeutic results or to prevent glutathione deficiency. Glutathione synthetase (GSS) deficiency is a rare, autosomal recessive metabolic disorder that prevents the production of glutathione. The GSS gene is located on the long (q) arm of chromosome 20 at position 11.2. More than 20 mutations in the GSS gene have been identified in people with glutathione synthetase deficiency. Cytosolic and membrane-bound forms of glutathione S-transferase are encoded by two distinct supergene families. These enzymes function in the detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress including lipid peroxidation[30]. The genes for SOD production are located on chromosomes 21, 6 and 4. Thus any genetic aberration involving these genes or chromosomal aberrations involving chromosome 20 and 21 that suppress the formation of these antioxidant enzymes will lead to excess populations of free radicals in the

body as found in people with Down's syndrome (trisomy 21) and it is the consequential detox problem that leads to oxidative stress that shortens lifespan in people with such aberrations.

Mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma[31], acceleration of age-related muscle mass loss[32], early incidence of cataracts and a reduced lifespan. Mice lacking SOD2 die several days after birth due to massive oxidative stress[33]. Mice lacking SOD3 do not show any obvious defects and exhibit a normal lifespan[34] but in humans it poses an increased risk of cancers because humans and apes lost the glucoronic pathway during the course of evolution and cannot produce vitamin C to form glucose unlike mice. Natural vitamin C can quench the superoxide and protect the cell from its toxicity.

Synthesis of antioxidant enzymes requires ATP and coenzyme Q10. Oxidative stress that leads to a decline in ATP output and/or oxidative injury to coenzyme Q10 or blocks the formation of this enzyme as for instance by drugs like statins, will hinder the formation of other antioxidant enzymes in the body. Lipid-lowering drugs, such as the "statins" (lovastatin, pravastatin, and simvastatin) and gemfibrozil, as well as oral agents that lower blood sugar, such as glyburide and tolazamide, tend to decrease serum levels of coenzyme Q10 and reduce the effects of coenzyme Q10 supplementation[35]. Beta-blockers (drugs that slow the heart rate and lower blood pressure) can inhibit coenzyme Q10-dependent enzyme benefits and biochemical pathways[36]. The contractile force of the heart in patients with high blood pressure can be increased by coenzyme Q10 administration[37]. Coenzyme Q10 and glutathione when taken with natural vitamin C can decrease insulin requirements in individuals with diabetes for the simple reason that this intervention biochemically repairs the oxidatively injured glucose and insulin molecules while coenzyme Q10 repairs the cell membranes.

Normally most of the coenzyme Q10 is in reduced form (ubiquinol, QH2), which is the form that is most effective as an antioxidant. The ubiquinol (QH2) can neutralize a lipid peroxyl radical by donating one of its hydrogen atoms to become the free-radical semiquinone (.Q-), which is then restored to a non-free-radical state by the respiratory chain Q cycle. QH2 or .Q- can also regenerate (recharge) the Vitamin E tocopheroxyl radical by electron donation[38]. Natural vitamin E is not produced in the body and must be taken through the diet. So, an enzyme that is produced in the body can recharge an antioxidant produced in plants. It represents a dimension in which one L-form antioxidant works in a network fashion with another that is exogenous but works within the natural antioxidant defense mechanism of the body. Vitamin E can also be recharged by glutathione and alpha-lipoic acid.

Co-Q10 is also vital for the formation of elastin and collagen. The side effects of Co-Q10 deficiency include muscle wasting leading to weakness and severe back pain, heart failure,

neuropathy and inflammation of the tendons and ligaments, often leading to rupture[39]. In healthy heart cells, coenzyme Q10 is about 9-10 times more abundant than in other muscles to enable the heart to function round the clock and its depletion in the heart cells can lead to cardiovascular problems.

Without Co-Q10, the cell's mitochondria are inhibited from producing energy from ATP, leading to muscle pain and weakness. The heart is especially susceptible because it uses so much energy[40]. Of the nine controlled trials on statin-induced Co-Q10 depletion in humans, eight showed significant Co-Q10 depletion leading to decline in left ventricular function and biochemical imbalances[41]. Coenzyme Q10 is an integral part of the pathway that uses ATP to release energy for use in the cell and any inhibition or block in this energy utilization can lead to fatigue, muscle pain and muscle fatigue. Low levels of coenzyme Q10 lower the repair of cell membranes and loss of integrity of cell membranes leads to disease conditions as it impairs mineral uptake and transport of macro-molecules across the oxidatively damaged membrane.

People who also take other drugs, including recreational drugs that deplete minerals and glutathione are at a very elevated risk of mitochondrial problems leading to symptoms associated with chronic oxidative stress and AIDS. Imagine the hundreds of millions of precriptions of statin drugs and other drugs that interfere with the body's natural antioxidant system. The fundamental flaw in modern pharmaceutical therapy is that its drugs increase the superoxide and hydrogen peroxide production in cells, which depletes the natural antioxidants. Excessive or long-term use of these drugs can lead to the production of secondary radicals that lead to the development of disease conditions. Many of these drugs are inhibitors or blockers that inhibit or block the formation of molecules like glucose or cholesterol. And they can inhibit or block other steps in healthy pathways that yield useful biomolecules. Blocking glucose production in diabetics, for instance, will lead to a lower production of antioxidant enzymes like glutathione. Sharp declines in glutathione yields can lead to cell death which means the correct therapeutic approach is to promote cell repair and promote glucose utilization in cell metabolism.

Is the pharmaceutical benefit of blocking the formation of cholesterol worth the risk of inhibiting and blocking the production of energy and antioxidants in the body? Put it another way, would anyone knowingly want a pharmaceutical benefit in exchange for a health risk or elevating a health risk? Yet, modern medicine, especially the block buster drugs, is all about inhibitors and blockers of biochemical pathways. And how would evolution reduce the reliance on selenium unless an alternative antioxidant that is not a seleno-protein has evolved to replace the role of gultathione?

Cholesterol production begins with acetyl-CoA, a two-carbon molecule sometimes referred to as the "building block of life" and the process uses three acetyl-CoA molecules that are combined to form six-carbon hydroxymethyl glutaric acid (HMG). The step from HMG to mevalonate

requires an enzyme, HMG-CoA reductase. Statin drugs work by inhibiting this enzyme and are referred formal to as HMG-CoA reductase inhibitors. All the potential numerous side effects of statin drugs arise from this inhibition because it is not an inhibition of the production of cholesterol alone, but an inhibition of a whole set of related biomolecules that are very useful to the body, including coenzyme Q10. Cholesterol is a very useful biomolecule that is used to make new cell membranes and hormones. Its depletion is not the cause of disease conditions but it becomes a risk factor in the development of disease only when it is oxidatively damaged. Oxidatively injured cholesterol molecules (LDL) cannot enter cells and they cannot enter biochemical pathways wherein it is used to make other biomolecules such as natural steroids but participates in plaque formation in the arteries initiating at sites of low grade infection and low grade inflammations. Hence, its levels in the bloodstream rise. Glutathione and fat-soluble antioxidant enzymes prevent the oxidative injury to cholesterol and other lipid molecules and promote their entry into cells and their utlization in biochemical pathways. High levels of blood antioxidant levels prevent low grade inflammations in the arteries and in other tissues.

ATP production also requires the production of acetyly-CoA. Since coenzyme Q10 is also involved in this important role within the mitochondria to yield the ATP molecule and later in its utlization to release energy for use in cells to produce other useful biomolecules, this role in the production of ATP in the citric acid [Krebs or tricarboxylic acid (TCA)] cycle must be briefly mentioned to understand its importance.

Each pyruvate molecule produced by glycolysis is actively transported across the inner mitochondrial membrane, and into the matrix where it is oxidized and combined with coenzyme A to form CO2, acetyl-CoA and NADH[42]. The acetyl-CoA is the primary substrate that enters the citric acid cycle. The enzymes of the citric acid cycle are located in the mitochondrial matrix, except succinate dehydrogenase, which is bound to the inner mitochondrial membrane as part of Complex II[43]. Reactions in the citric acid cycle oxidize the acetyl-CoA to carbon dioxide and yields reduced cofactors. In this reduction process, three molecules of NADH and one molecule of FADH2 are reduced to yield electrons that are a source of electrons for the electron transport chain (ETC) and also yield a molecule of GTP that is readily converted to an ATP molecule[44]. Many drugs can interrupt or block this ETC as well and can disrupt the healthy biochemistry within the mitochondria and cause dysfunction leading to the development of mitochondrial disease conditions including fatigue, chronic fatigue, myopathies and cancers.

It can be seen that within the mitochondria, the biochemical reactions proceed by generating electrons and by the transfer of electrons to yield new biomolecules including ATP for energy in cells. The human body, at this level, is in fact a nano biochemical system that is driven by natural antioxidants that can readily donate electrons and scavenge the superoxide produced as a

byproduct of this nano biochemical system and can scavenge free radicals within this system whereas drugs and chemicals can disturb it and disrupt it, causing impairment in the mitochondria and dysfunction of mitochondria leading to the development of disease conditions.

Inhibition in the yields of cellular energy must be given its due importance in the development of disease conditions because it is essential to the production of all the useful biomolecules in the body including collagen, elastin, HDL, antioxidant enzymes, hormones, genetic molecules and repair proteins and antibodies and in the generation of other immune responses.

Taking adequate amounts of natural vitamin C helps to boost this protective role of fat-soluble antioxidants as natural vitamin C can donate electrons to glutathione and alpha-lipoic acid that can, in turn recharge fat-soluble antioxidants, including natural vitamin E. Evolution has engineered, by some intelligent design and natural selection, biochemical pathways and integrated them into a complex whole. It includes the bio-engineering of selenium together with the other minerals, inlcuding copper, iron, zinc and managanese into a biologically active antioxidant system that works in a network fashion. Hence, generating a pharmaceutical benifit such as glucose-lowering or cholesterol-lowering while inhibiting or blocking the production of useful biomolecules that are fundamentally essential for health needs reexamination.

The seleno-protein called glutathione has an essential and fundamentally critical role in healthy biochemistry and is an integral part of the mammalian antioxidant network system that has evolved over the last 65 million years while plants have been around very much earlier. The earliest plant cells probably appeared about 900 million years ago.

In mammals there are three forms of superoxide dismutase – SOD1 functions in the cytoplasm, SOD2 in the mitochondria and SOD3 functions in the extracellular space. SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reactive centre. Low amounts of these minerals will hinder the formation of the three forms of SOD and leave the body open to problems caused by superoxide toxicity. The biochemical roles of these natural antioxidant enzymes have remained unaltered throughout evolution. No new biochemistry or new biochemical reactions evolved to replace the basic detox mechanism that converts the reactive superoxide into oxygen and hydrogen peroxide and then converting the hydrogen peroxide into water and oxygen. This biochemistry is responsible for health and when glutathione levels decline in cells below a certain critical level, apopotosis occurs and the cell dies. There is nothing to demonstrate that the plant or mammalian or human biological system has evolved alternative biochemical pathways that convert hydrogen peroxide into water and oxygen and there is certainly no evolutionary selection against selenoproteins or glutathione and there is, consequently, no reduction in the reliance on selenium for the production of selenoproteins.

On the other hand, there are factors that favor the natural selection of selenium. One of them, as discussed above, is that L-form selenium is an antioxidant by itself and secondly, selenoproteins are an integral part of the natural antioxidant network system. They protect the genetic material from oxidative stress and when in excess they can participate in DNA repair and prevent chromosomal aberrations. While glutathione and SOD promote healthy cell division and prevent oxidative stress and prevent cancers, another factor in favor of L-selenium is its anti-viral property.

In an outbreak involving 80 patients, oral sodium selenite at 2 mg. per day for 9 days was used to achieve a dramatic reduction in the overall mortality rate, which fell from 38% (untreated control group) to 7% (Se treatment group), thus giving an 80% reduction in mortality[45]. Using selenium at a dose of about 13 times the RDA as the sole therapy to effectively treat hemorrhagic fever is all the more striking in light of the fact that, according to conventional medical science, there is no effective treatment for hemorrhagic fever (viral infections with Ebola-like symptoms) not involving Ebola virus. There are a number of different hemorrhagic fever viruses, and they may share common mechanisms[46].

Dr. Gerhard Schrauzer has been accumulating evidence for the potential benefits of selenium for many years, not only against cancer, but also in viral diseases (and retroviral diseases in particular)[47]. High levels of glutathione and antioxidant enzymes might also have a repressive effect on viral replication, because it is known that oxidative stress (e.g. H2O2 exposure) that activates the breaking of the actin-viral particle bond can explain the cause of latency diseases. The pathology of muscle weakness and myopathy and chronic fatigue which are symptoms of AIDS is clearly associated with selenium deficiency[48]. Dr. Marianna Baum of the University of Miami has reported such Se abnormalities in several papers and she showed that IV drug users have low serum selenium that is 15 times more significant than low CD4 count as a risk factor for mortality[49] that clearly indicates that oxidative stress is the factor that compromises and impairs the immune system and opens it for disease conditions and infections to occur.

In conclusion, it can be said there is no "evolved reduced reliance on selenium" in the mammalian or human biological system. In fact, selenium intake is important for the formation of adequate amounts of seleno-proteins including glutathione. Glutathione is a critical component of the SOD enzyme reaction that scavenges the superoxide formed during cell metabolism to yield oxygen and hydrogen peroxide that is then converted into life-giving molecules – water and oxygen through the combined function of the glutathione-catalase pathway. It is a part of the miracle of life that protects cells from hydrogen peroxide toxicity and prevents the formation of the hydroxyl radical and no other antioxidant has evolved to take its place or the place of seleno-proteins in the mammalian biological system.

Many drug toxicities are mediated through mitochondrial damage and are associated with mitochondrial DNA depletion and certain commonly prescribed drugs lead to coenzyme depletion that can precipitate a problem in the amount of cellular energy to fuel healthy biochemistry. Selenium supplementation from bioavailable sources is necessary for most patients to recover from glutathione depletion. Antioxidants from green leafy vegetables are also required for recovery from coenzyme Q10 and alpha-lipoic acid depletion and to restore mineral depletion caused by antibiotics and chemo-drugs. Such selenium supplementation from dietary sources must be taken together with fruit and vegetable juices to augment the natural antioxidant network system as a whole and for the optimum functioning of the immune system which is necessary after any drug therapy.

Nutritional antioxidants have been suggested to play a role in cardiovascular disease prevention but what about their role in treating the disease? In a study, researchers investigated in vivo changes in rat arterial blood pressure induced by acute exposition to an increased load of peroxyl radicals and by the administration of selected antioxidants after chemically induced oxidative stress. Hydrosoluble and liposoluble peroxyl radicals, generated by 2,2'-azobis-(2-amidinopropane) dihydrochloride and 2,2'-azobis 2,4-di-methylvaleronitrile, induced a dose-dependent decrease in rat blood pressure. All antioxidants tested (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, vitamin C, glutathione and dithiothreitol) returned peroxyl radical-induced hypotension to normal. Of the various antioxidants tested, glutathione was the most effective in restoring blood pressure after peroxyl radical generation. The results suggest that acute exposition to peroxyl radicals has a hypotensive effect on blood pressure. Antioxidants inhibited peroxyl radical-mediated hypotension with glutathione being the most effective[50]. The mechanism involves direct quenching of superoxide and increasing intracellular levels of SOD and glutathione and catalase in endothelial cells to prevent the formation of the peroxynitrite oxidant (ONOO-) that binds nitric oxide when the superoxide reacts with the nitric oxide formed in endothelial cell lining as a result of which it is unavailable for vaso-regulation in the smooth muscles of arteries.

One of the very informative studies on the interventional effects of fruits and vegetables was undertaken by researchers in Denmark, Sweden, Netherlands and Belgium. This 25-d intervention study with complete control of dietary intake was performed in 43 healthy male and female nonsmokers who were randomly assigned to 1 of 3 groups. The fruit and vegetables (Fruveg) group received 600 g fruit and vegetables/d in addition to the basic diet; the Placebo group received a placebo pill, and the Supplement group received a vitamin pill designed to contain vitamins and minerals corresponding to those in 600 g fruit and vegetables. Biomarkers of oxidative damage to protein and lipids and of antioxidant nutrients and defense enzymes were determined before and during intervention. Glutathione peroxidase activity increased in the

Fruveg group only and although plasma lipid oxidation lag times increased during intervention in the Fruveg and supplement groups, the increase was significantly higher in the Fruveg group[51] **showing the better health benefit from fruits and vegetables over vitamin pills that may have synthetic ingredients.**

The progression of atherosclerosis in adults can be slowed down or halted by treatment with nutritional supplements [52]. The European Prospective Investigation into Cancer and Nutrition-Oxford study of 56,000 British men and women also demonstrated a reduced risk for coronary heart disease in vegetarians[53]. These effects are primarily due to the role of antioxidants in reducing or preventing oxidative damage and promoting healthy biochemistry which means the effects are, in fact dose dependent and dependent on age and serum antioxidant levels. Testing for serum antioxidant levels will become important in clinical nutrition before administering antioxidant interventions in order to asess or determine the dosages required for beneficial and the resultant therapeutic effects.

Elevated C-reactive protein (CRP) and plasma total homocysteine (Hcy) have been identified as risk factors for cardiovascular disease. "In the future, (life) underwriters may insist on a test for homocysteine because up to 25% of heart attacks are non-cholesterol related[54] and such risk is mediated through oxidatively damaged homocysteine molecules that can cause oxidative damage to cell wall membrane of heart cells and impair its integrity.

Micronutrients from plants may have beneficial cardiovascular effects. Phytonutrient concentrates can induce several favorable modifications of markers of vascular health in the subjects. A study on phytonutrient concentrate[55] supports the notion that plant nutrients are important components of a heart healthy diet because it showed that:-

1. systolic and diastolic blood pressure decreased significantly (–2.4 ± 1.0 mmHg, $P < 0.05$ and –2.2 ± 0.6 mmHg, $P < 0.001$, and

2. large artery compliance improved significantly (1.9 ± 0.6 ml mmHg–1 x 100, $P < 0.01$),

3. the progression of coronary artery calcium score was smaller than expected compared with a historical database ($P < 0.001$), and

4. laboratory testing showed a significant decrease in homocysteine ($P = 0.05$) and HDL cholesterol ($P = 0.025$). (The decrease in HDL may be due to a decrease in LDL and vlDl?)

5. There was also a a significant increase in beta-carotene, folate, Co-Q10 and -tocopherol (all $P < 0.001$).

A powder concentrate containing numerous phytonutrients, as opposed to tablets of single vitamins, was sufficient to attain the favorable surrogate results demonstrated with an improvement in markers of antioxidant status, homocysteine and glycosylated hemoglobin[56]. Numerous antioxidants and bioavailable minerals are always a better intervention to support the role of the antioxidant defense mechanism that better promotes healthy biochemistry, including:-

1. The repair of oxidatively damaged cell membranes,

2. Biochemical repair of oxidatively damaged biomolecules by electron transfer,

3. Prevention of formation of secondary free radicals and the peroxynitrite,

4. Improvement in the yields of ATP, antioxidant enzymes, repair proteins or antibodies etc, and

5. Improvements in the yield of cellular energy.

Glutathione, the tripeptide of glycine, glutamic acid, and cysteine (gamma-glutamyl-cysteinyl-glycine) which is involved in oxidation-reduction reactions, the conjugation of foreign substances for excretion and the transport of some amino acids into cells[57] is vital in the nano biochemical system within the mitochondria. Increasing the availability of circulating glutathione will provide a therapeutic benefit as well as a health benefit especially to patients who have low serum glutathione levels. Decreased glutathione levels are also a risk for cell cytotoxicity and cell death[58]. However oral administration may not be effective in increasing circulating glutathione levels, suggesting that the systemic availability of glutathione is negligible in man, because of hydrolysis of glutathione by intestinal and hepatic gamma-glutamyltransferase. Dietary glutathione is not a major determinant of circulating glutathione, and it is not possible to increase circulating glutathione to a clinically beneficial extent by the oral administration of a single dose of 3 g of glutathione[59] excepting the consumption of milk thistle and maca or sylimarin.

The high electron-donating capacity (high negative redox potential) of glutathione **combined** with high intracellular concentration (millimolar levels) generate great reducing power [60]. This characteristic underlies its potent antioxidant action and enzyme cofactor properties, and supports a complex thiol-exchange system, which hierarchically regulates cell activity[61]. The reducing power of ascorbate helps conserve systemic GSH[62] and any glutathione intervention for therapetic effects ought to include ascorbate from edible substances.

For rapid increases in circulating antioxidant levels and to increwase intracellular antioxidant enzyme levels, nano extracts from edible substances rich in glutathione, vitamin C and other antoxidants that are rapidly absorbed through the dermis will have wider application including in emergency room (ER) medicine.

This article forms a chapter in the book titled Fenton Medicine.

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