Full-Spectrum Antioxidant Therapy

Minimizing the Contribution of Oxidative Stress to Disease and Aging

Mark F. McCarty,

NutriGuard Research, Inc.,

markfmccarty@gmail.com

Section Headings

Abstract 3

Introduction 5

Spirulina and Phycocyanobilin – Getting to the Heart of Oxidative Stress 6

A Central Role for NADPH Oxidase in Oxidative Stress and Pathology 7

Why is Bilirubin so Protective? 8

Spirulina Can Pinch-Hit for Bilirubin! 11

Mitochondria – Another Key Source of Oxidant Stress 16

Astaxanthin – A Champion Antioxidant for Mitochondria and Cellular Membranes 17

Coenzyme Q10 – Mitochondrial Antioxidant and Bioenergy Cofactor 21

Coping with Peroxynitrite 24

Dr. Oster and High-Dose Folate 25

Vindication! 27

The Tremendous Trio of Antioxidant Protection 31

Boosting Uric Acid with Inosine 32

Allopurinol versus Xanthine Oxidase 34

Antioxidant Enzyme Induction 35

Green Tea Catechins 37

Melatonin as an Antioxidant 39

N-Acetylcysteine Boosts Glutathione 40

The Vitamin/Mineral Antioxidants 42

Carotenoids Afford Protection from Light-Generated Oxidants 48

Polyphenols Confer Vascular Protection 50

Boosting Nitric Oxide as a Complement to Antioxidant Measures 53

AMPK Activators – Metformin and Berberine 54

Potassium – Antioxidant Electrolyte for Vascular Protection and Bone Health 55

Protective "Carninutrients" with Antioxidant Potential 57

Glycine – Anti-inflammatory and Antioxidant Amino Acid 60

Controlling Iron Stores 62

Caloric Restriction and Vegan Diets vs. Mitochondrial Oxidative Stress 65

Promoting Mitochondrial Biogenesis as an Antioxidant Strategy 66

Uncouplers as Mitochondrial Antioxidants 67

Risk Factor Control – Statins and Angiotensin Antagonists 67

Oxidative Stress and Longevity 68

Resources 69

Appendix – A Few More Protective Nutraceuticals 71

References 83

Abstract

Oxidative stress clearly plays a mediating role in many pathologies and in some functional decrements of aging, but clinical evaluations of antioxidant supplementation have so far yielded rather lackluster results. However, there is reason to suspect that this reflects the limited antioxidant efficacy of the regimens evaluated, and that clinically important benefits may be achievable with a more rational and insightful choice of agents that exploits functional complementarities. This essay reviews the range of nutraceutical antioxidant options available, and proposes a comprehensive strategy incorporating the following elements: NADPH oxidase can be down-regulated with spirulina or phycocyanobilin-enriched spirulina extracts – an effect which mimics the physiological protection afforded by bilirubin. Astaxanthin, by preventing oxidant-mediated structural damage to the mitochondrial inner membrane, may limit the up-regulation of mitochondrial superoxide production seen in many pathologies. High-dose folic acid has potent antioxidant activity in tissues which concentrate folates, and may have particular merit for controlling the pathogenic impact of peroxynitrite-derived radicals. Inosine, by boosting levels of its metabolite uric acid, may limit the damage mediated by peroxynitrite in oxidant-driven CNS disorders (unlikely to respond to high-dose folate). Induction of many antioxidant enzymes and amplification of glutathione synthesis can be achieved with clinically effective phase 2 inducers such as lipoic acid and green tea catechins, and with nocturnal melatonin administration. Glutathione synthesis can be boosted further by optimizing the availability of its rate-limiting substrate with supplemental N-acetylcysteine or cystine. A comprehensive regimen encompassing most or all of these elements in clinically meaningful doses can be expected to have broad and potent clinical utility, and may be designated "Full-Spectrum Antioxidant Therapy". Ancillary strategies with antioxidant potential are also discussed here, including coenzyme Q10, the retinal xanthophyll carotenoids, polyphenols, potassium-rich diets, "carninutrients" (carnitine, creatine, taurine), glycine, phlebotomy, calorie restriction, vegan diet, and measures which promote mitochondrial biogenesis. Certain drugs can also aid control of oxidative stress in certain circumstances – allopurinol via inhibition of the pro-oxidant enzyme xanthine oxidase, and statins and angiotensin II antagonists via down-regulation of NADPH oxidase activation. Complementing full-spectrum antioxidant therapy with measures which amplify production of nitric oxide in the moderate physiological range – e.g. aerobic exercise training, quercetin/epicatechin, citrulline, metformin or berberine, and dietary nitrate – may potentiate the favorable impact of antioxidants on vascular health and dementia prevention, while also promoting bone density.

Introduction

It is widely acknowledged among medical researchers that excessive oxidative stress is a key mediator of a vast array of diseases, and is also a cause of many of the functional decrements that accompany aging. Yet controlled clinical trials of "antioxidant therapy" – usually involving just vitamin E, beta-carotene, or vitamin C – have so far yielded rather paltry, often disappointing results. This may reflect the fact that the antioxidants chosen for these studies have rather limited impact on intracellular oxidative stress and its metabolic consequences, at least in persons whose baseline nutrition is reasonably decent. However, recent biomedical discoveries may make it feasible to achieve truly effective control of oxidative stress, using nutrients, foods, and phytochemicals that are currently available. This essay sets forth a proposal for a **Full-Spectrum Antioxidant Therapy**, in which the remarkable antioxidant potential of spirulina and its key phytochemical phycocyanobilin is complemented by a number of other effective antioxidant measures.

What is proposed here is not a fixed regimen, but rather a general concept that can be tailored to the needs of individual people. For logistical reasons of cost or convenience – and, in the case of inosine, safety – it may not be feasible for a given person to employ all of these agents. And an individual's specific health needs should of course be taken into consideration in the choice of a supplementation regimen. Moreover, the term "Therapy" is used here loosely, inasmuch as this strategy may be appropriate for healthy people who wish to remain that way.

Ideally, Full-Spectrum Antioxidant Therapy should incorporate these key features:

- **Partial suppression of NADPH oxidase activity** by ingestion of **spirulina** or **phycocyanobilin**-enriched spirulina extracts;

- **Moderating mitochondrial oxidant generation while promoting optimal mitochondrial function** with supplemental **astaxanthin** and, in some circumstances, supplemental **coenzyme Q10**.

- **Scavenging of peroxynitrite-derived radicals** by supplementation with **high-dose folate** and, optionally, **inosine** or dietary nucleic acids;

- Induction of antioxidant enzymes and promotion of glutathione synthesis with **phase 2-inducing nutraceuticals –** most notably **alpha-lipoic acid** and **green tea catechins**-nocturnal **melatonin** supplementation, and **N-acetylcysteine**;

- Insuring adequate intakes of **nutritionally essential antioxidants** such as selenium, vitamin C and gamma-tocopherol with appropriate **nutritional insurance supplementation**.

Spirulina and Phycocyanobilin – Getting to the Heart of Oxidative Stress

To understand why spirulina has such exciting potential for coping with disorders associated with oxidative stress, we must first examine the physiological antioxidant role of **bilirubin**. Bilirubin is derived in the body from the breakdown of heme, an organic molecule that contains complexed iron and enables hemoglobin to carry oxygen; heme is also a component of many other vital enzymes. When heme is present in excess, an enzyme known as **heme oxygenase-1** (HO-1) cleaves it, generating three derivatives: a free iron atom, carbon monoxide, and biliverdin. An enzyme called biliverdin reductase, found in all mammalian cells, then rapidly converts biliverdin to bilirubin.

The mention of carbon monoxide understandably may raise some anxiety; in excess, this compound is a poison that can asphyxiate people whose heaters malfunction. But in the low concentrations generated by normal metabolism, it has a benign regulatory impact in our cells, and in fact can mimic some of the protective effects of the signaling molecule nitric oxide – both it and nitric oxide regulate cell behavior by activating soluble guanylate cyclase, which catalyzes cyclic GMP production.

But the most intriguing factor generated by HO-1 activity is bilirubin. Bilirubin is extremely insoluble; the liver conjugates it to glucuronic acid so that is becomes sufficiently soluble to excrete in the bile. When people with liver disorders develop jaundice, the yellowish pallor of their eyes and skin reflects the high circulating levels of conjugated bilirubin in the blood which the damaged liver has failed to excrete. But bilirubin is much more than just an excretory product; when generated within cells, it has a very potent antioxidant activity. Indeed, that's why HO-1 is considered to be an important antioxidant enzyme.

By definition, oxidative stress is characterized by an excess of unstable compounds known as free radicals, and other unstable molecules – such as peroxides – which they can give rise to, and wish in turn can generate the hydroxyl free radical. Free radicals are unstable because they contain unpaired electrons, and therefore are highly prone to grab another electron from another molecule, or to donate an electron to another molecule. (Chemical compounds are most stable when they contain paired electrons.) Most biological antioxidants act as scavengers – when they encounter a free radical, they readily donate an electron to the radical, generating a more stable compound. This of course converts the antioxidant into a free radical – but antioxidants are characterized by the fact that they are fairly stable in free radical form, and therefore won't steal electrons from other stable molecules. Moreover, cells have mechanisms for converting physiologically essential antioxidants – such as vitamin C, vitamin E, and glutathione – back to their native forms after they have donated electrons to free radicals. So scavenging antioxidants have the potential to defuse dangerous free radicals, protecting cellular proteins, fats, and nucleic acids from structural damage.

For many years, it was presumed that the potent antioxidant activity of the bilirubin generated within cells by HO-1 activity reflected its ability to scavenge free radicals. Bilirubin is indeed an efficient scavenger of a wide range of free radicals, and the radical scavenging activity of the

free bilirubin bound to albumin in the blood stream contributes importantly to the antioxidant activity of the blood. But the notion that the bilirubin within cells is acting primarily as a free radical scavenger has frankly never made sense. Here's why: most cells contain relatively high (millimolar) concentrations of the effective radical scavengers vitamin C and glutathione. In contrast, the concentrations of bilirubin generated within cells by HO-1 activity are in the low nanomolar range[1] – in other words, a concentration *over ten thousand times lower* than those of glutathione and vitamin C. The rate at which scavenging antioxidants can defuse free radicals is proportionate to the concentration of the antioxidant; since the inherent capacity of bilirubin to donate electrons is not vastly higher than that of vitamin C or glutathione, it is readily seen that the scavenging activity of intracellular bilirubin will be almost negligible compared with that provided by vitamin C and glutathione. So why does generation of bilirubin via HO-1 activity have such a physiologically important antioxidant impact? Recent research has provided a satisfying and exciting answer.

The fundamental source of most other free radicals in biological systems is a "progenitor" free radical known as **superoxide**. Superoxide is merely molecular oxygen (O2) with a single electron added to it. The chief fates of superoxide are to be converted to **hydrogen peroxide** and molecular oxygen – a reaction catalyzed by the enzyme superoxide dismutase – or to react spontaneously with nitric oxide to generate the very dangerous and unstable compound **peroxynitrite**. The hydrogen peroxide generated from superoxide, when present in very low concentrations, has a benign signaling function within many cells, reversibly altering the structure of cellular proteins by interacting with free sulfhydryl groups. But in excess it can lead to cell death or dysfunction, either by overdriving certain pro-inflammatory or cytotoxic signaling pathways, or by reacting with free iron or copper atoms to produce the vicious oxidant **hydroxyl radical**. Peroxynitrite, and other radicals derived from it, can have a range of adverse effects that we will discuss later. By donating an electron to iron or copper ions, superoxide can help to drive the production of hydroxyl radicals (the so-called "Haber-Weiss reaction").

Various enzymes and enzyme complexes within cells can produce superoxide by adding a single electron to molecular oxygen. During normal healthy metabolism, mitochondria – often called the "power plants" of the cell, because they generate large amounts of the energy catalyst molecule ATP – steadily produce small amounts of superoxide which are readily disposed of by antioxidant enzyme activity. However, when mitochondria become structurally disrupted in certain ways, or when they are "burning" excessive amounts of fuel, they can produce superoxide at an accelerated rate, and this may give rise to damaging oxidative stress.

A Central Role for NADPH Oxidase in Oxidative Stress and Pathology

Another key source of superoxide – and the most prominent source in many disease states – is an enzyme complex known as NADPH oxidase. (This complex actually occurs in several distinct isoforms;[2] it is not crucial to go into the details of this now.) Concentrations of NAPDH oxidase are especially high in white cells of the immune system that function as phagocytes, engulfing and killing bacteria and other microorganisms. When phagocytes ingest bacteria,

NADPH oxidase becomes activated, and the resulting production of oxidative stress within phagocytic vacuoles helps to kill the ingulfed bacteria. Indeed, people in whom the phagocytic form of NADPH oxidase is genetically absent are said to have chronic granulomatous disease, and suffer from recurrent infections owing to their impaired capacity to kill certain types of bacteria. However, forms of NAPDH oxidase are found in many other types of cells, including cells that don't participate in immune defense. In these cells, moderate activation of NAPDH oxidase generates hydrogen peroxide, and thereby can act in various ways to modulate cellular behavior in a physiologically appropriate way.

But ongoing medical research is demonstrating that, in a remarkably high proportion of health disorders, NADPH oxidase becomes *overactivated* in affected tissues, and the resulting oxidative stress either exacerbates or even mediates the disorder. Here is a *partial* list[3] of the disorders in which overactivity of NADPH oxidase is now believed to play a key pathogenic role:

Atherosclerosis / Hypertension / Cardiac Hypertrophy / Congestive Heart Failure / Aortic Aneurysms / Sleep Apnea / Tissue Damage stemming from Heart Attack or Stroke / Insulin Resistance Syndrome / Major Complications of Diabetes, including Kidney Failure, Blindness, and Heart Disease / Erectile Dysfunction / Cartilage Loss in Osteoarthritis and Rheumatoid Arthritis / Osteoporosis / Inflammatory Carcinogenesis / Alzheimer's Disease / Parkinson's Disease / Liver Cirrhosis associated with Hepatitis or Alcoholism / Sun-Induced Skin Damage and Sunburn / Pulmonary Fibrosis / Periodontal Disease / Pre-eclampsia / Asthma / Allergies / Septic Shock / Scleroderma / Glaucoma-induced Blindness / Sickle Cell Anemia

This no doubt is only a partial list, because there are other common disorders, such as macular degeneration and cataracts, which clearly are linked to increased oxidative stress, but in which the source of this oxidative stress has not yet been clearly defined.

As if this list weren't impressive (or depressing) enough, there is also evidence that NAPDH oxidase is chronically activated in many human cancers, and the resulting oxidative stress, by boosting growth factor activities, makes the cancer more aggressive, growing quicker and spreading more rapidly.[4]

And the adverse effects of cigarette smoke on the vascular tissue, and on other tissues distant from the lungs, appear to be mediated largely by reactive compounds that trigger NADPH oxidase activation.[5-8]

Clearly, whereas a little bit of NADPH oxidase activity is physiologically appropriate, excessive activity is very bad news indeed!

Why is Bilirubin so Protective?

So what does any of this have to do with bilirubin?

Simply this: Medical researchers have recently established that ***the physiological antioxidant role of bilirubin within cells reflects its ability to act as a very potent inhibitor of NAPDH oxidase activity***.[9-11] (The isoform specificity of this effect requires further clarification.)

This in turn provides a very satisfying explanation for the antioxidant role of HO-1. When cells are exposed to excess oxidative stress, this triggers increased production of HO-1. This increase in HO-1 activity accelerates the conversion of cellular heme to, among other things, bilirubin; the increase in bilirubin then acts to suppress NADPH oxidase activity which, in a high proportion of circumstances, is the key source of the cell's excessive oxidative stress.[12] Clearly, the induction of HO-1 represents a physiological feedback mechanism that helps keep oxidative stress in check.

This perspective makes it clear why bilirubin is very different from scavenging antioxidants like vitamins C or E. Here's an analogy that should make this concept easier to grasp:

Visualize a sink, with the tap jammed open. Water is spilling out into the sink; the sink is now full, and water is spilling out onto the floor. Think of the water on the floor as excessive oxidative stress. Scavenging antioxidants function like mops. Some mops work on one part of the floor, others work on another. None of the mops, by itself, can clean the whole floor.

What does bilirubin do? It turns off the tap! In other words, **bilirubin goes right to the source of the oxidative stress, turning it off**, and preventing *all* of the downstream consequences of excessive oxidative stress.

This latter consideration is very important. Antioxidants such as vitamin C and vitamin E can indeed dispose of some free radicals, but they do little to prevent the generation of hydrogen peroxide from superoxide. An excess of hydrogen peroxide is a key mediator of cell dysfunction and death in many disorders – which likely explains why supplementation with vitamins C and E hasn't been notably successful in many controlled clinical studies. Bilirubin functions at a fundamentally higher level.

These considerations suggest an important question: do moderate increases in bilirubin availability influence disease risk in humans? Recent genetic and epidemiological research suggests that the answer is yes.

Humans often inherit slightly different forms of genes; these genetic variations are known as polymorphisms, and a specific form of a polymorphic gene is known as an allele. The gene for HO-1 is polymorphic, and some alleles of this gene are considered "high expression"; in people who carry these alleles of HO-1, an oxidative stress induces a higher expression of HO-1 than in a person who carries a low expression allele. Perhaps not surprisingly, genetic studies are showing that people who carry one or two high-expression alleles of HO-1 are at lower risk for certain disorders: coronary artery disease, emphysema (in smokers), restenosis after angioplasty or coronary stenting, abdominal aortic aneurysms, lung cancer (in smokers), and oral cancer (in

betel nut chewers).[3, 13] No doubt this is just the beginning of genetic research into the health impacts of HO-1 polymorphisms.

Perhaps the most intriguing study in this regard was conducted by Japanese researchers, who noticed a remarkable phenomenon: when they segregated Japanese women by age, and looked at the extent to which these women carried the high expression alleles of HO-1, they found that the high expression alleles were much more common in elderly women than in younger women.[14] Does this mean the that the Japanese people have undergone remarkably rapid evolution in the last few decades? Not likely! No, the likely explanation is that the Japanese women who carried the low expression alleles tended to die off before they could become elderly! In other words, efficient induction of HO-1 increases average longevity. In light of what we now know about bilirubin and NADPH oxidase, it seems likely that efficient bilirubin production is largely responsible for this phenomenon.

Another polymorphic gene that influences bilirubin level codes for the enzyme UDP-glucuronosyltransferase 1A1 – more humanely abbreviated as UGT1A1. This is the liver enzyme that hooks bilirubin to a glucuronic acid so that bilirubin can be excreted through the bile ducts. A low expression allele of this gene is fairly common in humans, and people who inherit two copies of this low expression allele have a moderate impairment of their capacity to conjugate bilirubin; as a result, free bilirubin levels in their blood are 2-3-fold higher than in other people. People who for genetic reasons maintain relatively high serum bilirubin levels (about 20 μM) are said to have Gilbert syndrome (in honor of the French physician who first characterized it), and they typically carry two copies of the low-expression form of UGT1A1. Despite the fact that these people are said to have a "syndrome", there so far are no known adverse health consequences associated with it. (It can however be inconvenient, as physicians sometimes subject patients with Gilbert syndrome to batteries of liver tests, suspecting that the elevation of bilirubin may reflect liver disease!)

In fact, recent studies have shown that people with Gilbert syndrome are at decidedly lower risk for coronary heart disease and colorectal cancer.[15] And a remarkable recent Japanese study concluded that, in long-term diabetics, the patients who also had Gilbert syndrome were only about a third as likely to experience major common complications such as kidney failure, blindness, and coronary disease.[16] (It is no coincidence that the Japanese physician who organized this study, Dr. Toyoshi Inoguchi, has devoted much of his career to demonstrating the key role of overactive NADPH oxidase in the induction of diabetic complications.)[17]

Even in people who don't have Gilbert syndrome, relatively elevated serum bilirubin levels have been correlated with decreased risk for a number of health disorders, including coronary heart disease, lung cancer, chronic obstructive respiratory disease, diabetic complications, and rheumatoid arthritis.[18-23] And in striking concordance with the Japanese HO-1 study cited above, Dr. Laura Horsfall and colleagues found that serum bilirubin correlated inversely with risk for total mortality.[20]

(However, somewhat surprisingly, there is recent evidence that the low expression form of UGT1A1 is not associated with reduced disease risks.[24, 25] What this probably means is that increased blood bilirubin *per se* is not *mediating* the protection associated with high blood bilirubin levels. Rather, these increased levels may be a *marker* for the greater propensity of HO-1 to generate bilirubin within the body's cells. The levels of bilirubin which effectively inhibit NADPH oxidase within cells may be notably higher than serum levels of free bilirubin – unless those serum levels are exceptionally high. People with the very high serum bilirubin that characterizes Gilbert syndrome not only carry the low expression form of UGT1A1, but also tend to generate bilirubin at an increased rate within tissues; this can be deduced from the fact that the average serum bilirubin in people expressing the low-expression forms of UGT1A1 is only about 12 µM[26] – notably lower than the 20 µM traditionally required for a diagnosis of Gilbert syndrome.)

At this point, it has probably occurred to some thoughtful readers: why not just give people bilirubin supplements? Unfortunately, the extreme insolubility of bilirubin would make it hard to achieve effective bilirubin absorption. However, there is an easy way around this: biliverdin is much more soluble, is partially absorbed, and can be converted to bilirubin in the body. Indeed, a few rodent studies now show that oral biliverdin has potent antioxidant activity in rodents.[27]

But the problem with biliverdin is that it is extremely difficult and expensive for organic chemists to synthesize, and there are no known concentrated natural sources of it.

An alternative way to increase bilirubin levels would be to administer a drug or nutraceutical that very potently inhibits UGT1A1 activity in the liver – thereby driving serum bilirubin levels high enough to suppress cellular NADPH oxidase activity; this strategy has been dubbed "iatrogenic Gilbert syndrome".[3] There are indeed some drugs which have this activity, and clinical work exploring this approach is just beginning; doses of the HIV drug atazanavir sufficient to increase serum bilirubin 9-fold have been reported to favorably influence the function of vascular endothelium in diabetics.[28] A nutraceutical which achieved the same thing would be more useful from the standpoint of prevention – but, aside from the fact that silibinin, in very high and expensive doses, can raise bilirubin levels a bit,[29] there is little present evidence that nutraceuticals have practical potential in this regard.

There is however another option that should have great practical utility.

Spirulina Can Pinch-Hit for Bilirubin!

Spirulina is a cyanobacterium – also often described as blue-green algae, though scientists formally consider it a member of the bacteria family – that is one of the most ancient organisms in existence, and that has long been used as a human food. When the Spanish conquistadors first encountered the Aztecs, they observed the Aztecs harvesting wild-growing spirulina from the surface of Lake Texcoco (the lake which once surrounded what now is Mexico City); the Aztecs were ingesting the spirulina in various food products. Africans living near Lake Chad

have similarly been harvesting and eating wild-growing spirulina for centuries. Spirulina is an exceptional source of protein, and is rich in many micronutrients and phytochemicals, notably carotenoids such as zeaxanthin. Within the last several decades, spirulina grown in specially constructed ponds has been promoted and sold as a "health food" in the U.S. and elsewhere - though most people who have used it have taken it in capsule form, as its odor and taste are less than pleasing to most people.

A distinctive feature of spirulina – which it shares with various other microalgae, though not the popular Chlorella – is that it is very rich in a protein called **phycocyanin**. Phycocyanin can constitute up to 20% of the dry weight of spirulina. The reason why spirulina make so much of this protein is that it functions – much like chlorophyll – as a light harvester. In other words, it absorbs light energy and makes it available for the metabolic needs of the organism. But the portion of phycocyanin that actually absorbs light is not protein, but rather a so-called chromophore – a deep-blue organic compound known as **phycocyanobilin** that is covalently bound to the phycocyanin protein. (Scientists typically abbreviate "phycocyanobilin" as "PCB", but we prefer to use "**PhyCB**", to avoid confusion with polychlorinated biphenyls, the environmental contaminants that are also commonly abbreviated as "PCB".) The attached PhyCB imparts such a deep blue color to phycocyanin that this protein has been approved for use as a food dye (in case you need blue food!)

But here's what makes PhyCB exceptionally interesting to us: it is almost identical in structure to biliverdin! In fact the only differences occur at the far ends of the molecules, as you can see in Figure 1 below. The reason these two molecules are so homologous in structure is that algae make PhyCB from biliverdin; the small modifications at the ends of the molecule make it feasible to attach PhyCB to the phycocyanin protein. Although there is relatively little biliverdin in spirulina, PhyCB can constitute about 0.6% of the dry weight of the organism, reflecting its exceptionally high level of phycocyanin.

And here's another interesting fact. You will recall that our cells have an enzyme, biliverdin reductase, that converts biliverdin to bilirubin. Back in the early 1990s, algae experts at the University of California, Davis, examined the impact of biliverdin reductase on PhyCB, and discovered that it very efficiently converted PhyCB to a novel compound which they named phycocyanorubin.[30]

So the obvious question is: can phycocyanorubin, like bilirubin, inhibit NADPH oxidase?

After receiving a tiny sample of purified PhyCB, and purchasing some biliverdin, Dr. Inoguchi set out to answer this question. Using three different types of human cells in culture, he activated the NADPH oxidase in these cells, and then observed the impact of adding either biliverdin or PhyCB to generation of oxidative stress in these cultures. He was pleased to discover that PhyCB worked virtually as well as biliverdin in quelling oxidative stress in these

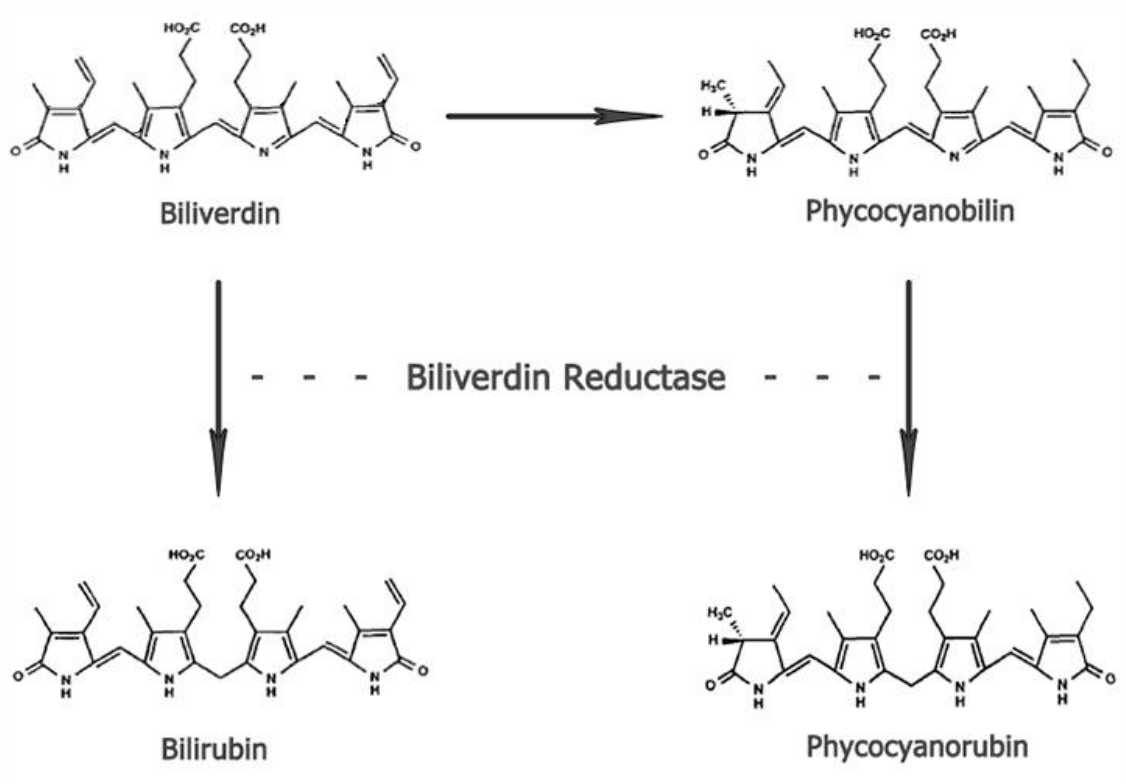

Figure 1

cells, inhibiting NAPDH oxidase activity in a clearly dose-dependent manner. (We presume, though, that it was actually bilirubin and phycocyanorubin, generated by biliverdin reductase activity within these cells, that directly inhibited NADPH oxidase; Dr. Inoguchi chose to work with biliverdin only because it is much more soluble than bilirubin, and thus much easier to work with.)

These findings evidently raise the possibility that PhyCB might be used as a nutraceutical inhibitor of NAPDH oxidase. But is PhyCB absorbed intact when spirulina or phycocyanin is ingested, in a form that can exert antioxidant activity? There so far is no direct evidence on this point – but a raft of indirect evidence strongly suggests that the answer is yes.

There are in fact dozens of studies in rodents – most conducted by scientists in Cuba, India, or Mexico – demonstrating that oral ingestion of phycocyanin exerts a very broad range of anti-inflammatory, antioxidant, and cytoprotective effects.[31, 32] Since dietary protein is almost totally broken down to its constituent amino acids during the process of digestion, it is logical to suspect that PhyCB or some metabolite thereof is responsible for these protective effects. And every model in which oral phycocyanin has proved effective is one in which NADPH oxidase plays a key role in the pathology.

So draw your own conclusions!

To get an idea of the scope of this research, you can take a look at Table 2 from a review article that summarizes the rodent studies that Cuban scientists have conducted with oral phycocyanin.[31] It cites 12 different models in which phycocyanin has exerted anti-inflammatory or cell-protective effects. Most intriguinely, one these studies demonstrates protection of the brain. Other research, from Mexico, has shown that oral administration of whole spirulina is substantially protective in a mouse model of Parkinson's disease.[33] The likely implication of this is that PhyCB has access to the brain – a non-trivial consideration, given that the brain is protected by a blood-brain barrier that prevents many molecules from entering it. Since oxidative stress – most of it derived from NADPH oxidase – is believed to play a key role in causing the nerve cell death and dysfunction that characterize many neurodegenerative disorders,[34, 35] including Alzheimer's, Parkinson's, and ALS, the ability of PhyCB to penetrate the brain may have exciting implications for the prevention and possibly even control of these tragic afflictions.

Until recently, no one had thought to test oral phycocyanin in animal models of metabolic diseases such as atherosclerosis and diabetes – disorders in which NADPH oxidase overactivity is a key mediator. But soon after Dr. Inoguchi conducted his cell culture studies with PhyCB, a French research group reported the observation that oral administration of either phycocyanin or whole spirulina to cholesterol-fed hamsters exerts a profound anti-atherosclerotic effect, inhibiting the early stages of atherosclerosis (fatty streaks) by over 80%.[36] Even more recently, Dr. Inoguchi has observed that, in genetically obese diabetic mice, feeding either biliverdin, PhyCB, or phycocyanin largely prevents diabetic kidney damage – decreasing the urinary loss of albumin, and effectively eliminating oxidative stress and the sclerotic response in the glomeruli.[27, 37] (Progressive sclerosis of the glomeruli is what ultimately causes kidney failure in diabetics.) These new findings evidently accord very nicely with Dr. Inoguchi's previous observation that diabetic complications are considerably rarer in patients with Gilbert syndrome.

Intriguingly, other recent research suggests that the biliverdin/bilirubin generated by HO-1 activation in lymphocytes can promote the generation of T-reg cells, lymphocytes which play a crucial role in prevention and control of autoimmune and allergic disorders.[38-42] And there is now evidence that PhyCB may have the potential to mimic this effect as well.[43] Moreover, there is reason to suspect that this effect is *not* mediated by antioxidant activity[44, 45] – hence implying that bilirubin has another key physiological target (other than NADPH oxidase) in cells, as some researchers have suggested.[46] Hence, biliverdin/bilirubin have anti-inflammatory effects that are independent of, but presumably often complementary to, their antioxidant actions. In this regard, administration of both bilirubin and of phycocyanin has been shown to be markedly effective in experimental autoimmune encephalomyelitis, the standard mouse model for multiple sclerosis.[43, 47, 48] These considerations suggest that PhyCB may have potential for preventing or treating the entire range of autoimmune and allergic disorders – and also for preventing transplant rejection, a phenomenon that has been demonstrated in rodents treated with biliverdin or bilirubin.[49, 50] On the other hand, high T-reg activity can impede anti-

cancer immunity, and may also compromise resistance to infection, so it seems likely that high intakes of PhyCB may prove to be contraindicated in certain clinical circumstances. But it should also be noted that NADPH oxidase activation boosts the growth of certain cancers, and mediates life-threatening complications of infections such as septic shock; indeed, both biliverdin and phycocyanin have been shown to control septic shock in rodents.[9, 51, 52] Hence, the overall impact of PhyCB in cancer or infection is not readily predicted, and must ultimately be addressed in controlled clinical trials.

Efforts to develop PhyCB-enriched spirulina extracts as a nutraceutical antioxidant are now underway, and hopefully will ultimately achieve success. In the meantime, though, everyone is free to use intact spirulina. So-called "health foods" more often than not fail to live up to their promotional hype when subjected to rigorous clinical evaluations – but spirulina may ultimately prove to be a very notable exception to this rule.

But what intake of spirulina will provide meaningful protection? Since there has so far been very little sophisticated clinical research with spirulina or phycocyanin, all we can do at present is make an educated guess, based on the presumption that absorption and metabolism of PhyCB in humans is roughly similar to that in rodents. One such assessment has concluded that 1-2 rounded tablespoons daily (about 15-30 grams) would likely replicate the substantial antioxidant benefits observed in rodent studies – though it is quite conceivable that lower intakes will also provide some meaningful protection.[32]

How much spirulina or PhyCB would be too much? You will recall that, in immune cells, NADPH oxidase plays a key role in the killing of ingested bacteria. And this enzyme complex also contributes to metabolic regulation in many types of cells. So literally wiping out NAPDH oxidase activity would be a very bad idea. And boosting T-reg activity may be inadvisable in certain circumstances. Fortunately, we can take comfort in the fact that people with Gilbert syndrome don't seem to have any evident health problems – if they are more prone to infections, no one has noticed it yet, and they seem functionally normal in other respects. Nor have any evident problems been described in people who ingest spirulina regularly. And rodents fed diets containing as much as 30% spirulina appear to thrive[53, 54] – in particular, this doesn't harm the reproductive process[55, 56] (good news in light of the fact that overactivity of NADPH oxidase is a mediator of the common pregnancy syndrome pre-eclampsia).[57] This likely implies that a moderate reduction of NADPH activity usually has benign health impacts, perhaps in part because immune cells have complementary mechanisms for killing bacteria. So the degree of NADPH oxidase suppression associated with Gilbert syndrome or the consumption of feasible amounts of whole spirulina doesn't seem to present a problem – but can provide some important health protection. Once pure PhyCB is available in supplemental form, rodent studies should enable us to assess how much is too much. One comforting fact is that bilirubin is cleared from the body rather quickly. If the same holds for PhyCB, it should be possible to quickly restore normal NADPH oxidase activity by abstaining from supplementation for a few days.

Mitochondria – Another Key Source of Oxidant Stress

NADPH oxidase is one of the two chief sources of excess oxidative stress that promote tissue damage and disease; the other is the mitochondrion (plural mitochondria), a sausage-shaped structure that is found in most cells – commonly known as the "power plant" of the cell. Mitochondria are rather like cells-within-cells, as they have their own DNA and are membrane encapsulated; they may actually have evolved from ancient bacteria. Mitochondria are known as power plants because they are the primary source of the bioenergy catalyst ATP; this molecule provides the biochemical energy required to drive tens of thousands of enzymatic reactions in which biological molecules are synthesized, transformed, or transported. Without ATP, a cell could only survive for seconds; that's why properly functioning mitochondria are crucial for most cells.

How do mitochondria make ATP? The details are extraordinarily complex, but it is feasible to sketch the rough outlines of this process. Essentially, mitochondria produce high energy electrons in the process of metabolizing sources of food energy – primarily glucose and fatty acids. These high energy electron are then passed down a sort of bucket brigade known as the electron transport chain (ETC). As the electrons flow down this chain, they progressively lose energy – but some of this energy is conserved through the synthesis of ATP; this flow of electrons is said to be "coupled" to ATP synthesis. At the foot of the transport chain, the electrons are added to molecular oxygen, generating water molecules. In the absence of oxygen, the electrons can't flow, and ATP isn't generated – that's why cells that rely on mitochondria for their ATP can't survive long without oxygen.

As electrons flow down the ETC, a few of them get sidetracked and are added to molecular oxygen midway in the chain, generating the radical superoxide (rather than water). Under normal circumstances in healthy cells, this is the fate of only 1-2% of the electrons flowing down the ETC, and the modest amount of superoxide generated can be coped with by the antioxidant enzymes of the cell. However, under certain circumstances, the rate at which mitochondria generate superoxide is greatly amplified. This happens, for example, when glucose-permeable cells are exposed to too much glucose (as in diabetes); this boosts the rate at which mitochondria generate high energy electrons, resulting in a commensurate increase in superoxide production. Excess levels of oxygen can likewise accelerate this process. Paradoxically, a sudden reduction in oxygen availability (hypoxia) also accelerates mitochondrial generation of superoxide, presumably because excessive numbers of electrons build up in the ETC.[58, 59] And inflammation can boost mitochondria superoxide production, as a key pro-inflammatory hormone (tumor necrosis factor-alpha) decreases the production of certain protein components of the ETC, impairing the efficiency of electron flow.[60]

A perverse peculiarity of mitochondria is that, when they are damaged by oxidative stress, the structural changes that occur increase the propensity of mitochondria to generate superoxide.[61] In other words, oxidant stress begets oxidant stress! Key proteins in the ETC are particularly prone to being damaged by peroxynitrite, and the fats in mitochondrial membranes are readily

peroxidized. In mitochondria that have been damage by oxidants, the flow of electrons down the ETC is less efficient, a higher proportion of these electrons leak out to generate superoxide, and the coupling mechanism linking electron flow to ATP generation is impaired (a phenomenon known as "uncoupling"), so that ATP production falls off while oxidant production goes up. To put it succinctly, mitochondrial electron flow becomes slow, leaky, and uncoupled. Oxidative stress can also cause mutations in the mitochondrial DNA, ultimately resulting in the production of mutant mitochondrial proteins that may be functionally deficient. In some disease states, overactivated NADPH oxidase provides the intial oxidative stress that turns mitochondria into self-sustaining sources of oxidants.[61, 62] Thus, mitochondria can act as amplifiers of the oxidative damage triggered by NADPH oxidase. Conversely, the oxidants produced by mitochondria can promote activation or increased expression of NAPDH oxidase in some cells.[63] Hence, even when a stimulus which promotes oxidative stress is alleviated, vicious cycle mechanisms often can maintain the excess production of oxidants.

An important illustration of the principle that oxidative damage can turn mitochondria into potent generators of oxidative stress is the phenomenon of "ischemia-reperfusion damage" – a key mediator of the tissue damage caused by heart attack or stroke. This results when blood flow to a tissue is curtailed temporarily, and then re-established. Severe oxidative stress arises in the tissue after blood flow is restored, resulting in cellular death or dysfunction. Because of ischemia reperfusion damage, therapeutic measures which break down blood clots after a heart attack or a stroke are often not as beneficial as might have been hoped.

As noted, when tissues are suddenly deprived of blood flow and oxygen, superoxide production by the ETC *increases*, even though less oxygen is available for superoxide production. This is presumably because of the sudden back-up of electrons in the ETC, which are diverted to react with oxygen to generate superoxide at complex III of the ETC.[59, 64, 65] This superoxide is rapidly enzymatically converted to hydrogen peroxide, which, under these conditions, can then react with iron atoms to produce vicious hydroxyl radicals, the most reactive of free radicals. As a result, fats and proteins in the inner mitochondrial membrane that contains the ETC sustain severe structural damage; this damage impairs the ETC's capacity to sustain an efficient flow of electrons down the chain. As a result, when blood flow is restored and the tissue is reoxygenated, mitochondrial superoxide production rises dramatically, as more oxygen is now available to react with electrons "leaking" out of the damaged ETC. This burst of superoxide production during reperfusion is responsible for much of the tissue damage caused by heart attack or stroke; it also contributes to the adverse health impact of the temporary reductions in tissue oxygenation associated with sleep apnea or sickle cell crisis.

Astaxanthin – Champion Antioxidant for Mitochondria and Cellular Membranes

Remarkably, the best available natural antioxidant for mitochondria is a phytochemical produced in algae and cyanobacteria – just like PhyCB! Astaxanthin (AX) is an oxygenated derivative of beta-carotene; such derivatives are known as xanthophylls, and include the compounds lutein and zeaxanthin that function to protect the retina from light-induced oxidative

stress. But AX appears to have the greatest antioxidant activity of any of the xanthophylls. Like other xanthophylls, it is poorly water soluble, and tends to integrate itself into cellular membranes, with the oxygenated portions of the molecule (ketone and hydroxyl groups) sticking out into the cellular fluid. These oxygenated portions of the molecule are capable of donating electrons, and are largely responsible for AX's high antioxidant activity; indeed, AX's superiority as an antioxidant reflects the fact that it is more oxygenated than other xanthophylls.[66-69]

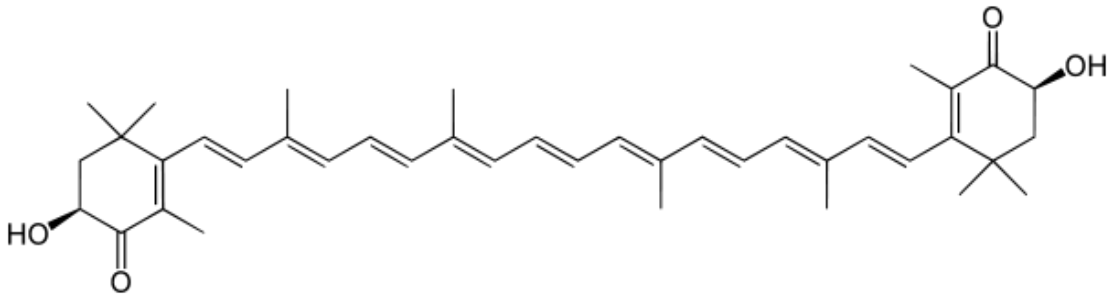

Figure 2. Astaxanthin

Some types of algae are rich natural sources of AX; the algae *Haematococcus pluvialis* can be 1-3% AX by dry weight, and is cultivated as a source of AX used in feeds and supplements.[70-72] AX is also found in animals which consume AX-rich algae, directly or indirectly. AX is found in many crustaceans, and is the source of the pink color in salmon and in flamingoes! Most humans have little AX in their diets – unless they a lot of salmon or shrimp – but humans can absorb AX efficiently and distribute it to their tissues. Whether AX can function as efficiently as lutein or zeaxanthin to protect the retina from light-induced oxidative stress is not yet known. But AX shares the ability of lutein and zeaxanthin to quench singlet oxygen (a damaging oxidant produced by blue light exposure, as discussed below), and in mice dietary AX gets into the retina and can help retinal photoreceptors survive certain stresses.[73-75]

AX appears to be more effective than any other natural antioxidant yet studied for protecting cellular membranes from oxidative damage. When cells are exposed to AX, a high proportion of it ends up in mitochondrial membranes, and many of the favorable effects of AX supplementation observed in rodent or clinical studies are likely attributable to its ability to prevent structural damage to mitochondria subjected to oxidative stress.[76-79] Not only does this help the mitochondria to keep functioning efficiently as sources of useable biochemical energy (ATP), but it also prevents the sorts of damage to the mitochondrial ETCs that can turn them into more potent sources of superoxide. Hence, AX serves the dual purpose of protecting cellular membranes from oxidative damage, and of restraining the mitochondrial superoxide production that gives rise to oxidant stress. And this raises the intriguing prospect that joint

administration of PhyCB and AX could help to control the two primary sources of cellular oxidative stress – NADPH oxidase and mitochondria.

A clear example of AX's potential as a mitochondrial antioxidant is provided by a number of studies showing that pre-administration of AX (or of a synthetic water soluble derivative of AX that can be administered by intravenous infusion, and is converted to AX in cells) can provide substantial protection for tissues subjected to ischemia-reperfusion.[80-82] This has been demonstrated in rats, rabbits and dogs whose coronary arteries have been temporarily occluded; the extent of heart muscle damage that results is reduced by up to 70% in animals pre-treated with AX. In rats, this compound likewise protects the brain and the liver from ischemia-reperfusion damage.[83, 84] The ability of AX to protect mitochondria from oxidative damage has also been demonstrated in a number of cell culture studies.[76-79] One of these studies suggests that AX might have potential for preventing Parkinson's disease, and possibly other types of neurodegeneration associated with oxidative stress;[78] indeed, a more recent mouse study shows that AX pre-treatment is markedly protective in the chief model for Parkinson's disease.[85] Another of these studies helps to rationalize a previous study in diabetic mice showing that oral AX can decrease the fibrotic kidney damage (glomerulosclerosis) caused by hyperglycemia.[76, 86] And AX has also been shown to help preserve the function of beta cells in genetically obese mice prone to diabetes.[87]

The only study to-date to examine the impact of AX on cholesterol-induced atherosclerosis found that AX failed to provide protection in cholesterol-fed rabbits.[88] This contrasts with the substantial protection afforded against atherosclerosis by PhyCB in cholesterol-fed hamsters,[89] and possibly reflects the fact that NADPH oxidase, rather than mitochondria, is the chief source of oxidative stress in vascular endothelial cells exposed to excessive levels of cholesterol-rich LDL particles.

Remarkably, mouse studies reveal that AX may have potential for promoting leanness. When mice are allowed to run on treadmills, pre-supplementation with AX enhances their endurance and promotes a more selective utilization of fat for fuel.[90, 91] The increase in endurance presumably reflects, in part, a sparing of glycogen stores, so that it takes longer for running mice "to hit the wall" when glycogen runs out. Not surprisingly, when mice were allowed to run 5 times weekly for 4 weeks, the AX-supplemented mice ended up with 16% less body fat (epididymal fat) than those not supplemented. Similarly, when mice are fed a high-fat diet that promotes obesity, Ax-supplemented mice gain less weight (despite comparable calorie intake) and burn fat at a higher rate throughout the day.[92] (This latter study may or may not be germane to humans, since mice have a type of fat that humans lack called brown fat, which can efficiently burn fat to generate heat.) The ability of AX to promote a more selective utilization of fat during exercise is remarkably parallel to a recent clinical study with spirulina supplementation; when volunteers were supplemented sequentially with a modest dose of spirulina (6 grams daily) and matching placebo, and were studied during standardized 2-hour runs on a treadmill, they burned 10% more fat (and 10% less carbohydrate) during the spirulina supplementation phase, than during the placebo phase.[93] (And endurance in a subsequent

maximum-intensity sprint was increased by the spirulina.) A reasonable interpretation of these findings is that the oxidative stress generated in muscle by prolonged exercise may selectively damage the capacity of mitochondria to utililize fat for fuel. Indeed, in one of the AX mouse studies, the researchers found that the enzyme which is rate-limiting for fatty acid uptake into mitochondria was oxidatively modified in exercised mice – but that chronic administration of AX alleviated the extent of this oxidative damage.[91] So perhaps the combination of PhyCB and AX will prove useful for endurance athletes (in whom fat is a major fuel), and in people who want to use exercise training to achieve weight control.

So far, AX hasn't been studied in rodent models of liver disease (though it did decrease liver fat levels in mice on a high-fat diet). There is considerable evidence that mitochondrial damage may be largely responsible for the hepatic oxidative stress that characterizes and promotes tissue damage in non-alcoholic fatty liver disease, now the leading cause of liver failure in the U.S.;[94-96] AX, in conjunction with other antioxidant measures, may prove to have considerable value in this disorder.

Until recently, there were so few properly controlled clinical studies with AX that is was hard to know what dose to recommend, or even to conclude that AX had practical potential for promoting human health – since most rodent studies have employed doses that would be ruinously expensive if extrapolated to humans. However, recent clinical studies with orally administered AX have demonstrated that daily intakes as low as 12-20 mg daily may have clinically worthwhile antioxidant activity in humans. The most important of these studies was a double-blind, placebo-controlled randomized trial in which healthy subjects with moderately elevated triglyceride levels received daily doses of 0, 6, 12, or 18 mg AX for 12 weeks.[97] The 12 and 18 mg doses were found to exert markedly favorable and statistically significant effects on parameters associated with metabolic syndrome – triglycerides fell by about 25%, whereas the protective hormone adiponectin rose by about 20%, and HDL also increased modestly; there was also a trend for a reduction in systolic blood pressure, but this did not achieve statistical significance. These findings stand as confirmation of previous uncontrolled clinical studies suggesting that comparable intakes of AX can have a favorable impact on parameters of metabolic syndrome.[98, 99] Inasmuch as rodent studies suggest that oxidative stress in fat cells is the underlying cause of metabolic syndrome,[100, 101] a reasonable interpretation of these findings is that long-term administration of adequate amounts of AX can suppress oxidant stress in fat cells, possibly by preserving mitochondrial structure. Similar beneficial effects have been reported in human diabetics supplemented with spirulina (8 grams daily).[102] This may mean that both NADPH oxidase and mitochondria contribute meaningfully to oxidative stress in overstuffed fat cells.

Also worthy of note is an earlier double-blind, placebo-controlled study in which 16 mg AX, administered daily for 3 months to men being treated for infertility, was associated with improved sperm function and a notable increase in pregnancy rates.[103] This provides further confirmation that 12-20 mg per day AX has the potential to confer clinically important antioxidant protection – a dose that is reasonably affordable at current prices.

By targeting the two primary sources of oxidative stress in most health disorders – and in the aging process – joint administration of PhyCB and AX may have truly remarkable potential for promoting health and graceful aging. Clinical research with these agents is still in its infancy – stay tuned!

Coenzyme Q10 – Mitochondrial Antioxidant and Bioenergy Cofactor

Most human diets presently provide only trivial amounts of AX. However, mitochondria naturally possess antioxidant enzymes and small molecule antioxidants which under normal healthy circumstances enable them to cope with modest levels of oxidants, so that they remain structurally and functionally intact. The most important small molecular antioxidants in this regard appear to be glutathione – we'll have much to say about this presently – and **coenzyme Q10** (CoQ). CoQ is also known as ubiquinone – the name stemming from the fact that the distribution of CoQ in the plant and animal kingdom is ubiquitous (you will find it in any organism that has mitochondria). CoQ, a fat-soluble molecule found primarily in the mitochondrial inner membrane, functions as a physiologically essential electron carrier in the ETC bucket brigade; without CoQ, mitochondria couldn't generate ATP. Adequate availability of mitochondrial CoQ helps to insure the efficiency of electron flow, helping to minimize superoxide production by the ETC. However, CoQ can also function as a scavenging antioxidant. When electrons have been added to CoQ, the molecule is known as "ubiquinol"; in this "reduced" form, CoQ can donate electrons to free radicals, quenching them. The oxidant scavenging activity of ubiquinol is versatile and important; in particular, ubiquinol – like tetrahydrofolates - can quench peroxynitrite-derived radicals, protecting the proteins of the ETC from peroxynitrite-mediated damage.[104-106] Ubiquinol also helps to prevent the peroxidation of the fats in mitochondrial membranes. The high utility of CoQ as a mitochondrial antioxidant reflects the fact, that after it has donated an electron to quench a free radical, the ETC "reloads" it with another electron, so that it is once again primed to scavenge oxidants.

On the other hand, CoQ also has pro-oxidant potential; after ubiquinol donates one electron (becoming a compound known as semiubiquinone), it is capable of donating another to molecular oxygen to generate superoxide. Indeed, excessive levels of semiubiquinone in the ETC of mitochondria appear to be a major source of mitochondrial superoxide production. So whether CoQ is anti-oxidant or pro-oxidant may depend on the circumstance.

When pathologies associated with mitochondrial damage are benefitted by CoQ supplementation, it is often unclear whether this benefit reflects an antioxidant effect, or rather increased availability of a crucial bioenergy catalyst that promotes more efficient mitochondrial ATP generation. Perhaps in some instances both mechanisms are at work. It should be recalled that the pioneer of coenzyme Q supplementation, Dr. Karl Folkers, judged the likely utility of clinical coQ administration by measuring the extent to which added CoQ would boost the activity of CoQ-dependent enzymes in the mitochondrial ETC of leukocytes or other tissues.[107, 108] In any case, it is clear that CoQ supplementation often has the potential to help mitochondria do their job more efficiently.

Although modest amounts of CoQ are found in most foods, the body can synthesize its own CoQ, so that a dietary source is not absolutely necessary. Nonetheless, CoQ supplementation can be employed to boost the CoQ content of mitochondria throughout the body. Not surprisingly, CoQ supplementation has been found to be beneficial – in rodent studies or clinical trials - in a number of disorders in which oxidative damage to mitochondria plays a key role.

The best established clinical use for supplemental CoQ is in the management of congestive heart failure – one of the most common causes of death and physical incapacity in elderly Americans; CoQ has been found to modestly boost the pumping action of the heart, likely by promoting effective mitochondrial function and ATP generation.[109-111] Since excess generation of peroxynitrite is known to be a key mediator of this syndrome – and proteins of the mitochondrial ETC are particularly prone to damage by peroxynitrite-derived radicals – it is reasonable to expect that supplemental CoQ could be beneficial in heart failure patients. The utility of CoQ in this syndrome is less notable in patients who are concurrently receiving treatment with ACE inhibitor drugs, now commonly used to treat congestive failure;[112] these drugs suppress the production of a hormone, angiotensin II, that can trigger oxidant damage to heart mitochondria by stimulating NAPDH oxidase activity. A recent study concludes that low blood levels of CoQ in patients with congestive failure predict decreased survival.[113] These considerations suggest that CoQ supplementation may be particularly prudent for patients experiencing heart failure. There is also some evidence that CoQ may help to prevent heart failure in cancer patients being treated with certain cardiotoxic chemotherapy drugs, such as adriamycin; these drugs can cause excessive oxidative stress in heart mitochondria, so CoQ's protective function in this regard may reflect its role as a mitochondrial antioxidant.[114] (The impact of AX on the cardiotoxicity of these drugs also warrants examination.)

Clinical trials, as well as rodent studies, indicate that supplemental CoQ can have antihypertensive activity. A recent meta-analysis concludes that, in placebo-controlled studies, the average response to CoQ was a 17 point drop in systolic pressure and an 8 point drop in diastolic pressure – a benefit as great as that imparted by most anti-hypertensive drugs, yet without any discernible side effects.[115] While the basis of this effect is still not entirely clear, recent rodent studies show that oxidative damage to neuronal mitochondria in key regions of the brain which regulate the sympathetic nervous system is a key mediator of blood pressure elevation in many models of hypertension.[61, 116-120] Injection of minute amounts of CoQ into these brain regions can alleviate mitochondrial damage, thereby preventing or correcting the rise in blood pressure.[61]

Other research indicates that CoQ may sometimes aid the protective function of the endothelial lining of arteries – likely reflecting a role for mitochondrial oxidant production in endothelial dysfunction.[121-123] In rodent studies, pre-administration of CoQ has a favorable impact on the damage to cardiac tissue evoked by a temporary cessation of blood flow;[124-126] this is not surprising given the prominent role of peroxynitrite and mitochondria-generated oxidative stress in ischemia-reperfusion damage..[127] And there is also recent evidence that CoQ may have a

modestly favorable effect on endurance, fatigue, and muscle damage in athletes, whose muscle fibers are subjected to increased oxidative stress during prolonged exertion.[128-131]

In many neurodegenerative disorders, neuronal dysfunction is characterized by impaired mitochondrial function and increased oxidative stress – suggesting possible utility for supplemental CoQ.[132] In fact, this nutrient has been shown to have a beneficial impact on rodent models of neurodegenerative disorders, most notably Parkinson's disease, in which neuronal mitochondrial function is known to be impaired.[132-135] The cerebral neurons that are damaged and killed in Parkinson's patients are known to be particularly susceptible to oxidative stress. Future clinical trials will be required to determine whether CoQ can help prevent or treat disorders such as Parkinson's, Alzheimer's, and ALS. Initial clinical trials with high-dose CoQ in Parkinson's patients suggest that the highest doses might slow functional decline.[136-139]

About a decade ago, a clinical trial from India reported that CoQ could improve insulin sensitivity in hypertensive patients; a recent rodent study likewise suggests that CoQ can favorably influence insulin resistance syndrome.[140, 141] In type 2 diabetics, some reports indicate that CoQ can aid vascular function and glucose control, whereas others fail to observe metabolic effects.[121, 142, 143]

Statins, drugs which aid cholesterol control, help prevent heart attacks, and may confer a range of other protective benefits, nonetheless can decrease blood and tissue levels of CoQ by interfering with its synthesis. Whether CoQ supplementation would improve long-term health outcomes in statin-treated patients still remains to be assessed; however, contrary to the hopes of some researchers,[144] such supplementation does not appear to prevent the statin-induced muscle damage that occasionally requires discontinuation of these drugs.[145, 146] Perhaps statin users who have congestive heart failure or hypertension would be most likely to benefit from supplemental CoQ.

CoQ supplementation is well tolerated, even in the very high doses (up to 3,000 mg daily) now being tested in patients with neurodegenerative disorders.[147] In dry form, it is inefficiently absorbed; special micellized preparations have improved availability, and thus may be preferable.[148, 149] Furthermore, recent research shows that ubiquinol, the reduced form of CoQ that functions directly as an antioxidant, is better absorbed than CoQ per se.[150] Thus, micellized forms of ubiquinol may be the most effective way to supplement with CoQ; such preparations are now commercially available. Relatively high doses may be advisable for patients who are coping with neurodegenerative diseases, to insure that adequate amounts get through the blood-brain barrier. In light of current evidence, it won't be surprising if future research establishes that CoQ is likely to benefit the range of disorders in which mitochondria are subjected to excessive oxidant stress, or in which tissue depletion of CoQ impairs the efficiency of mitochondrial function. Nonetheless, from the standpoint of providing antioxidant protection to biological membranes (including those of mitochondria), we judge that AX is likely to emerge as more cost effective.

Coping with Peroxynitrite

As we have noted previously, one of the key mediators of the tissue damage induced by oxidative stress is the compound peroxynitrite, which forms spontaneously from the interaction of superoxide with nitric oxide (NO). NO is an important signaling molecule in the vascular system and many other tissues. In the vasculature, moderate normal concentrations of NO promote vasodilation, and play a profoundly important protective role in warding off atherosclerosis, hypertension, heart attack, and stroke. Indeed, one of the key reasons why superoxide is toxic to the vascular system is that, by reacting with NO, it suppresses the protective bioactivity of this compound. But peroxynitrite is dangerous in its own right. Within body tissues, peroxynitrite reacts almost immediately with bicarbonate to produce an unstable compound that quickly breaks down to form nitrogen dioxide and carbonate radicals.[151] These highly reactive compounds viciously attack neighboring molecules – in particular, they degrade DNA, the genetic blueprint of our cells. This can give rise to mutations – indeed, genetic damage mediated by peroxynitrite-derived radicals is believed to be largely responsible for the increased cancer risk associated with chronic inflammation in certain tissues such as the stomach, colon, liver, or bladder.[152] When peroxynitrite-mediated DNA damage in a cell is extensive, this causes overactivation of the enzyme called PARP [poly(ADP-ribose) polymerase] that in turn can lead to catastrophic metabolic failure and cell death.[153]

Peroxynitrite forms whenever superoxide is formed in tissues that also generate NO. Modest amounts of NO are produced chronically in the vascular system and in neurons (nerve cells), but under inflammatory conditions, most tissues can form NO owing to increased expression of an enzyme known as inducible nitric oxide synthase, which has a very high capacity for NO generation. Since inflammation invariably is also associated with increase superoxide production, peroxynitrite is a major player in inflammatory disorders.

As we have discussed above, much of the tissue destruction that follows a heart attack or a stroke is caused by ischemia-reperfusion damage. When the reduction in blood flow to a tissue is only temporary, as it often is in these disorders – especially when patients have access to thrombolytic therapy that breaks down blood clots – much of the actually cell death that results occurs after blood flow is restored to the affected tissues. This is because rapid generation of superoxide – and thus oxidative stress – is dependent on an adequate tissue concentration of oxygen. When oxygenation is restored to tissues, increased production of superoxide and of vascular NO (which likewise is dependent on oxygen) leads to peroxynitrite generation, which is responsible for much of the subsequent tissue death. In rodent models of ischemia-reperfusion damage, administration of experimental drugs which degrade peroxynitrite provides important tissue protection.[154-156]

Peroxynitrite also plays a key role in the circulatory collapse and organ damage associated with septic shock[157] – a common cause of death in overwhelming infections – and it contributes to the loss of contractile power that compromises heart function in congestive heart failure[158] (responsible for hundreds of thousands of deaths annually in the U.S. alone). It also is a

mediator of the atherogenic process. In particular, peroxynitrite impairs the function of the key vascular enzyme, NO synthase, that generates protective NO, by damaging a cofactor – tetrahydrobiopterin - required for proper function of this enzyme.[159] In fact, not only does this decrease the capacity of this enzyme to produce NO, but it causes the enzyme to start producing superoxide – potentially further increasing peroxynitrite production! Another key vascular enzyme damaged by peroxynitrite is prostacyclin synthetase,[160] whose product prostacyclin helps ward off blood clots and promotes appropriate vascular dilation. So while peroxynitrite is a bad actor in many acute medical problems, it also works more insidiously over decades to degrade vascular health. And one of the most important ways in which superoxide contributes to neuronal death and dysfunction in neurodegenerative disorders is by giving rise to peroxynitrite, which is highly toxic to neurons.[35]

But why should we need to worry about peroxynitrite if PhyCB is available to suppress superoxide production? Well, for one thing, NADPH oxidase is not the only potential source of superoxide. Mitochondria produce superoxide, and this production is often amplified in stressed tissues. And other enzymes – notably xanthine dehydrogenase[161] and NO synthase[159] – can be structurally altered under stress so that they also generate superoxide. And even when NADPH oxidase is the predominant source of superoxide in a tissue, bear in mind that it is feasible and safe to achieve only a partial inhibition of this enzyme complex with bilirubin or PhyCB. Moreover, it is conceivable that some forms of the NADPH oxidase complex are not inhibitable by bilirubin/PhyCB. So medical strategies for coping with peroxynitrite are clearly required in any case.

Fortunately, a natural metabolite, uric acid (also referred to as urate), formed by the metabolic breakdown of nucleic acids (more specifically, the purine nucleotides), can act as a highly effective scavenger for peroxynitrite-derived radicals.[162] This suggest that strategies for raising tissue urate levels may be useful for controlling peroxynitrite-mediated disorders. We will discuss this option presently – but first we want to take a detour to tell a very bizarre story that may have major implications for peroxynitrite control.

Dr. Oster and High-Dose Folate

Dr. Kurt Oster was a German cardiologist who emigrated to the U.S. in the 1930s to avoid the Nazi horrors. He had a very individualistic mindset – some would call him crankish – but he also had acute observational powers. After the cholesterol-lowering advice then prevalent – "eat more unsaturated fats" – failed to prevent his own second heart attack, he began to study the pathology of atherosclerosis. He was one of the first to notice signs of oxidative stress in atherosclerotic arteries – and one of the first to grasp that oxidative stress played a mediating role in atherosclerosis. At the time – this was before it was known that NAPDH oxidase was expressed in vascular tissues – xanthine oxidase, an altered form of the healthy enzyme xanthine dehydrogenase, was known to be a potent producer of superoxide. Dr. Oster suspected that xanthine oxidase was the source of oxidative stress in atherosclerosis. But where did this xanthine oxidase come from? Oster evolved a theory that the process of homogenation makes

the xanthine oxidase in cow's milk absorbable – and that this absorbed xanthine oxidase was the culprit in atherogenesis.[163, 164] The fact that hardly anyone else believed this theory – most scientists were fixated on blood-borne cholesterol – did not deter this single-minded scientist.

If xanthine oxidase was responsible for atherosclerosis, it should follow that drug-mediated inhibition of this enzyme would be markedly protective. The drug allopurinol – which treats gout by inhibiting xanthine oxidase or its precursor xanthine dehydrogenase – had recently become available, and he tried it on several angina patients. While he judged that it was beneficial for this purpose (an observation recently confirmed in a controlled study, as we discuss below[165]), he worried that this then novel drug might have undesirable side effects in long term use. Besides, using a natural compound would seem more sensible for preventive purposes in healthy people. So Oster decided to study the use of high intakes of the B vitamin folic acid (a.k.a. folate).

During the 1930s, scientists had reported that folic acid could inhibit xanthine oxidase. We now know that these reports reflected an experimental error – the preparations of folic acid available at that time contained a contaminant that was responsible for the observed inhibition. But Dr. Oster did not know this. More recent studies have shown that folate intakes as high as 1,000 mg daily (over a thousand times the nutritional dose), though well tolerated, have no impact on gout, which is effectively treated by inhibiting xanthine oxidase.[166] This proved to be a case in which a scientific error may have serendipitously led to a key breakthrough!

Dr. Oster began to treat his heart patients with high daily doses of folic acid – 40-80 mg daily – and he was impressed with what he observed.[163, 167] He noted substantial improvements in angina pain; in particular his own consumption of nitroglycerin fell about twenty-fold. Patients who had intermittent claudication were able to walk more effectively. In diabetics who had ulcers that had failed to heal for months – owing to impaired circulation – healing occurred after folate therapy began. He even suspected that folate was reducing the incidence of heart attacks in his treated patients. Moreover, these high intakes of folate – a hundred-fold higher than the nutritrional range – did not cause any evident side-effects. He also made the incidental observation that high-dose folate seemed to be helpful in patients with psoriasis.[168]

Dr. Oster excitedly reported these observations at medical meetings and in medical articles. Dr. Kurt Isselbacher, a top Harvard cardiologist, spoke approvingly of Dr. Oster's work. But few of his colleagues took Oster seriously, mainly because they didn't believe his xanthine oxidase theory of atherogenesis; they had already concluded that high cholesterol was the key cause. (And it turns out that they were largely right – we now know that high levels of cholesterol-rich LDL particles in the bloodstream act on the vascular lining to activate NADPH oxidase – the true primary source of the oxidative stress that Oster suspected was behind atherosclerosis.[169, 170])

And well-intentioned meddling by the U.S. Food and Drug Administration soon made it difficult for other physicians to attempt to replicate Dr. Oster's observations. Although high intakes of folate are not directly toxic, they can mask the early signs of vitamin B12 deficiency

– a feature of the potentially deadly disease pernicious anemia, in which autoimmune attack on the stomach lining compromises B12 absorption; severe B12 deficiency is also sometimes seen in strict vegans, who avoid any dietary animal products (as plants don't make B12). When B12 deficiency leads to anemia, people usually seek medical attention, and physicians can readily diagnose B12 deficiency and correct it with B12 injections. But if a B12-deficient person is concurrently taking high doses of folic acid, this tends to prevent the anemia, so medical attention is not sought. This potentially can be a problem, because in the longer term, B12 deficiency can damage neurons – and this damage is not always reversible when B12 therapy is finally instituted. So there was at least a theoretical possibility that high intakes of folate could be harmful to people with pernicious anemia, by preventing early diagnosis. Accordingly, the FDA banned folate supplements providing more than 800 mcg (i.e. 0.8 mg) per daily dose. This action occurred at about the time that Oster was reporting his observations with high-dose folate. As a result, the only practical way for physicians to attempt a replication of Oster's work was to go to a chemical supply house for the raw chemical and make their own capsules. Few if any tried.

Dr. Oster spent much of the rest of his career inveighing against the cholesterol theory of atherogenesis – which did not enhance his credibility with his colleagues. He also made futile attempts to persuade the dairy industry to use higher processing temperatures that would have destroyed the activity of the xanthine oxidase in milk. Because Oster had become a "true believer" in folate therapy, he never undertook the placebo-controlled clinical studies that might have convinced his colleagues that he was on to something. He co-authored a popular book describing his observations and theories – "The XO Factor"[163] – and died in relative obscurity in the 1990s.

Vindication!

Long after Oster's death, several groups of scientists made an intriguing observation: intravenous infusions of the chief natural metabolite of folic acid – 5-methyltetrahydrofolate – rapidly improve the function of the inflamed vascular endothelium of people or animals who have atherosclerosis or diabetes.[171] More specifically, they found that this treatment boosted endothelial production of protective NO. Further studies demonstrated that 5-methyltetrahydrofolate was restoring the normal function of NO synthase in inflamed endothelial cells.[172-175] You will recall that this function is often compromised, because peroxynitrite induces oxidative damage to tetrahydrobiopterin, a key cofactor for this enzyme.

This puzzle was resolved several years ago by biochemists at Oxford University. They observed that various "reduced" forms of folic acid – forms which folic acid is converted to when it is taken up into cells, most notably 5-methyltetrahydrofolate – can act as highly effective quenchers for peroxynitrite-derived radicals.[176] Moreover, after a reduced folate molecule donates an electron to deactivate peroxynitrite-derived radical, enzymatic mechanisms in the cell can restore its reduced form – making it feasible for single molecule of folate to dispose of a great many radicals. In other words, within cells reduced folates can function much

like the physiological scavenging water-soluble antioxidants glutathione and ascorbate, which are cycled back to their reduced active forms after quenching radicals via electron donation.

But the key clinical advantage of folate is that, whereas intracellular concentrations of glutathione or ascorbate can be increased only marginally by oral supplementation strategies, high-dose folate supplementation has the potential to increase cellular levels of reduced folates by over an order of magnitude in endothelial cells, and likely a range of other tissues. Most cells contain a "reduced folate carrier" – a transport protein in their membranes that allow reduced forms of folate to diffuse in or out. The affinity of this carrier for reduced folate is about 1-5 µM – which means that this is the concentration which enables a half-maximal rate of transport.[177] When people take supplemental folate in doses of 5 mg or less, the blood levels of folate that are achieved – mostly in reduced form – are about 0.1 µM, or less than a tenth the level that would promote a maximal rate of intracellular uptake via the reduced folate carrier.[178] So when Oster chose to use 40-80 mg folate daily in his patients, he fortuitously was using a dose of folate that could be expected to markedly boost intracellular levels of reduced folates.

Researchers at Massachusetts General Hospital have confirmed that orally administered high doses of folic acid can indeed have a favorable impact on the heart. In patients with coronary heart disease, they found that an acute oral dose of 30 mg folic acid improves a phenomenon known as shear-induced vasodilation, which helps deliver oxygenated blood to heart regions that are underoxygenated.[179] The authors concluded that "it follows logically that high-dose folate may reduce the occurrence of ischemia in patients with coronary disease." This is of course precisely the observation that Dr. Oster had made in his patients with ischemia. Also noted was a modest drop in mean blood pressure –likely stemming from increased systemic NO bioactivity.

A subsequent Belgian study showed that, in patients who had recently suffered a heart attack, a 10 mg daily dose of folate boosted shear-induced vasodilation of the brachial artery.[180] As one of these researchers, Dr. An Moens, told me pithily, "We have shown that Dr. Oster was right!"

A recent rat study by Dr. Moens and colleagues has achieved an even more remarkable result.[181] They mimicked the ischemia-reperfusion damage produced by a heart attack by tying off a coronary artery in rats for 30 minutes, and then releasing it to reestablish flow. Some of the rats were treated with a high-folate diet in the days prior to this procedure – or received high-dose folate intravenously right before coronary flow was restored. In the days following these procedures, the rats were killed and the extent of damage to their heart tissue was determined. Incredibly, the folic acid pretreatment had reduced cardiac cell death by over 90%! Furthermore, the folic acid pretreatment had favorably influenced the bioenergy status of the heart during the period of ischemia, had reduced the incidence of heart rhythm disturbances (arrhythmias), and preserved the ability of the heart to generate nitric oxide.

The ability of high folic acid intakes to protect tissues subjected to a temporary loss of blood flow likely reflects the fact that peroxynitrite is a key mediator of cell death under these circumstances. The favorable effect on nitric oxide production likewise may reflect

peroxynitrite's adverse impact on nitric oxide synthase. It is less clear why folate aided preservation of bioenergetics during the ischemic period, since presumably little peroxynitrite was being formed during this time (owing to oxygen deficit). Not unlikely, the electron-donor capacity of reduced folates can quickly repair the oxidative damage to the ETC of mitochondria mediated by hydroxyl radical during the ischemic period.[58, 59]

The editorial in the journal Circulation which accompanied this pioneering study was entitled, "How does folic acid cure heart attacks?"[182] This title was of course consciously hyperbolic. In actual clinical experience, portions of heart muscle often die by necrosis because reperfusion is not achieved within 30 minutes, as it was in the Moens rat study. But this study provides compelling evidence that the significant proportion of cardiac myocyte death and dysfunction which can result from reperfusion injury can be substantially blunted by effective antioxidant measures - hence making prompt thrombolytic therapy a more compelling option.

Since NO functions physiologically as a vasodilator, it is logical to suspect that restoring effective NO production with high-dose folate could have a favorable impact on elevated blood pressure. Indeed, Oster observed blood pressure reduction in some of this folate treated patients, and Moens likewise reported a modest but significant reduction in average blood pressure in heart attack survivors treated with 10 mg folate daily. Now a study from Italy echoes these findings – significant reductions in both systolic and diastolic blood pressure, as well as improved insulin sensitivity, were observed in post-menopausal women treated with 15 mg daily of 5-methyltetrahydrofolate.[183]

We should make clear that these effects of high-dose folate have nothing whatever to do with reducing circulating levels of the compound homocysteine. If you follow the popular medical literature, you probably know that moderate elevations of the compound homocysteine, an amino acid metabolite, have been linked to increased risk for heart attack and stroke. People who have genetic abnormalities that lead to extremely high levels of homocysteine throughout life – a disorder known as hyperhomocysteinemia – clearly are much more prone to stroke or heart attack. (Indeed, at these high concentration, homocysteine activates NADPH oxidase in the vascular lining.[184]) Since modest supplemental intakes of folic acid – in the nutritional range – typically lower blood homocysteine levels, a number of studies have evaluated the impact of supplemental folate on heart attack risk in people with modest elevations of homocysteine. By and large, these studies have concluded that little if any benefit is achieved,[185] and it now seems likely that moderate increases in blood homocysteine are serving as a marker for a pro-inflammatory state that is the true inducer of increased heart attack risk. It's important to understand that the failure of nutritional intakes of folate to influence heart attack risk in these studies tells us *nothing* about the medical potential of the hundred-fold higher doses which Dr. Oster employed in his patients.

Now that we know that high-dose folate can act as an effective scavenger of peroxynitrite-derived radicals, it seems clear that Dr. Oster's clinical observations with it represent only the tip of the iceberg of folate's clinical potential.[186] Any situation involving ischemia-reperfusion

might potentially be benefited by high-dose folate. This includes not only heart attacks and strokes, but also chronic disorders such as sleep apnea and sickle cell disease in which intermittent deficits of oxygenation in specific organs or the whole body induce oxidative stress and tissue damage. The use of high-dose folate in septic shock and congestive heart failure evidently merits study. And Oster's observation that folate seemed to benefit patients with psoriasis suggests that it should be tested in inflammatory disorders involving increased production of both NO and oxidative stress.

For the acute care of emergencies such as heart attack, stroke, or resuscitation for hemorrhage (which also entails a temporary deficit of oxygenation in vital organs), it may be extremely fortunate that a reduced form of folate is already clinically available for intravenous administration. This is known as leucovorin (a.k.a. folinic acid) – it is used to control the toxicity of the widely-used cancer chemotherapy drugs methotrexate, and to boost the efficacy of the chemotherapy drug 5-FU. Administration of leucovorin, in conjunction with thrombolytic therapy at the first sign of a heart attack or stroke, or in conjunction with blood transfusion in hemorrhage victims, may well prove clinically valuable. In patients with severe infections, it might prove useful for aiding survival in threatened septic shock. These applications could all be readily tested in animal models; Dr. Moens' studies with simulated heart attacks in rats represent a pioneering effort in this regard.

Although most studies with high-dose folate have focused on the vascular system, folate has the potential to provide important antioxidant protection to any tissue capable of concentrating it against a gradient like endothelial cells do. Two recent rodents studies reveal that the liver may be a good target for high-dose folate's benefits; folate was shown to have a markedly protective effect on fatty liver disease induced by a high-fat diet in mice – a model for the "non-alcoholic fatty liver disease" commonly associated with obesity and insulin resistance syndrome, now the leading cause of liver failure in the U.S. – and also provided some modest protection to the liver of rats chronically fed ethanol.[187, 188] Evidently, the exploration of high-dose folate's antioxidant potential has just begun.

Before moving on, a word is in order regarding high-dose folate and cancer risk. There is currently some concern among cancer researchers that increasing the availability of folate may enable certain types of cancer cells to grow more rapidly, and hence hasten the onset and possibly promote the survival of incipient cancers.[189] On the other hand, it is recognized that when folate status is poor, pre-cancerous cells may be more prone to accumulate mutations, because folate is required for the production of thymidine, an essential component of DNA; when thymidine levels are low, the compound deoxyuridine can be incorporated into newly synthesized DNA in its place, and this can give rise to heritable mutations or DNA chain breaks. But the greater availability of thymidine when folate levels are ample may also enable some cells, including cancer cells, to multiply more rapidly. So it is thought that good folate status may help to prevent cancer by maintaining the fidelity of DNA replication, but might also increase cancer risk by supporting a maximal growth rate in incipient cancer.[190] Folate status also has the potential to influence cancer risk by modulating the frequency of DNA methylation,

which regulates gene expression. Fortunately, an overview of pertinent studies suggests that initiation of folate supplementation by middle-aged adults is not associated with increased cancer risk – with the possible exception that risk for prostate can may be modestly increased.[191] (The selective impact on the prostate may reflect the fact that prostate cells have a higher requirement for folate that most other tissues.[192, 193]) Whether lifelong folate supplementation might increase prostate cancer risk is not clear – as prevention of mutations over a lifetime might be expected to have a worthwhile protective effect. In this regard, Harvard researchers found that folate supplementation for 15 years or more was associated with a marked decrease in risk for colorectal cancer in women, whereas supplementation initiated more recently did not impact cancer risk, possibly because a more modest antimutagenic effect was counterbalanced by a stimulative impact on the growth of incipient cancers.[194]

With respect to high-dose folate supplementation, this may be exceptionally protective in regard to mutagenesis because reduced folates could be expected to protect DNA from pro-mutagenic peroxynitrite-derived radicals and perhaps other mutagenic radicals that mediate the oxidative DNA damage associated with chronic inflammation – an effect that should be complementary to the antimutagenic impact of ample thymidine availability afforded by folate supplementation. Indeed, the recent study showing that high-dose folate is protective in a mouse model of fatty liver disease found that the folate treatment decreased oxidative damage to hepatic DNA.[187] And, in regard to the impact of high-dose folate on cellular multiplication, it seems likely that high-normal physiological levels of folate would be sufficient to maximize this, and that higher levels of folate would not be expected to have a greater impact in this regard.[193] Hence, it seems unlikely that high-dose folate supplementation would have a more negative impact on cancer risk than the moderate doses in current use (i.e. 400-800 mcg daily) – and indeed might have a more favorable impact because of its profounder anti-mutagenic potential. Concern regarding cancer risk should not be an excuse for stifling the clinical research with high-dose folate that is long overdue.

The Tremendous Trio of Antioxidant Protection

Although, as we will discuss below, a great many additional antioxidant nutraceutical strategies have valuable potential for promoting or recovering health, I would argue that PhyCB, astaxanthin, and high-dose folate are all "superstars in waiting", that, particularly if used as a functional combination in clinically effective doses, can be expected to have a major favorable impact on the innumerable health disorders driven or exacerbated by oxidative stress. "In waiting", of course, because so far clinical researchers have shown limited interest in exploring their efficacy. (If they were patented artificial chemicals, you could bet that hundreds of millions would be expended in this effort!)

However, for this strategy to be optimally practical, some innovations will be required. If the optimal clinical benefits of PhyCB require ingesting 15 g or more of spirulina daily, it would be very helpful if nutraceutical entrepreneurs developed reasonably affordable spirulina extracts enriched in PhyCB, such that an effective daily dose could be provided in a few capsules.

Legal barriers to the use of high-dose folate need to be surmounted; they have no logical merit if ample B12 is administered concurrently. And hopefully further advances in the aquacultural production of astaxanthin will enable the inherent cost of this agent to decline a bit. In the meantime, nothing currently prevents laboratory researchers from evaluating this combination in rodent models of oxidant-driven pathologies.

Boosting Uric Acid with Inosine

Unfortunately, there may be one key drawback to the use of high-dose folate as a peroxynitrite scavenger – it appears unlikely that high oral intakes of folic acid will achieve marked increases of folic acid in brain tissue, owing to obstruction by the blood-brain barrier.[186] This is particularly unfortunate in light of the fact that peroxynitrite is believed to be a key mediator of common neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Whereas most of the body's tissues, relying on the reduced folate carrier, can increase intracellular folate levels in proportion to increases in blood levels of reduced folate, this does not appear to be true for the brain. The brain does indeed have vital need for folic acid, and the blood-brain barrier has a carrier mechanism that brings blood-borne folate into brain tissue. But the capacity of this carrier mechanism is saturated at modest blood concentrations of folic acid that prevail in normally nourished people; in other words, increasing blood levels of folic acid in such people won't meaningfully increase the rate at which the brain takes up folic acid. Moreover, direct injection of high-dose leucovorin into the cerebrospinal fluid of cancer patients has proved toxic – at least one patient died.[195] So folic acid doesn't appear to have potential as a peroxynitrite scavenger for the brain. (The possibility remains open, however, that high-dose folate might have access to the vascular endothelium of cerebral arteries – in which case it might aid stroke prevention and therapy.)

There is however an alternative possibility – albeit one that has some potential drawbacks. You will recall that uric acid is a natural metabolite of nucleic acids that can efficiently scavenge peroxynitrite-derived radicals. In fact, it is frequently employed as a peroxynitrite scavenger in cell culture studies. And it is feasible to increase blood and tissue levels of uric acid – including probably brain levels – by ingesting nucleic acids or dietary purines such as **inosine**. (Inosine-5-monophosphate is in fact an approved food additive, used as a flavor enhancer.)

It is intriguing to note that people with relatively high blood levels of uric acid – or diets rich in nucleic acids - appear to be at decidedly lower risk for Parkinson's disease.[196, 197] Moreover, a high intake of dairy products seems to increase risk for this disorder[198] – and, for reasons not yet clear, certain milk proteins tend to lower blood urate levels by boosting the efficiency with which the kidneys excrete urate.[199, 200] High blood urate is also linked to lower risk for multiple sclerosis[201] – and increasing urate levels by feeding mice inosine has been shown to be protective in experimental autoimmune encephalomyelitis, commonly viewed as a rodent model for human multiple sclerosis.[202] Indeed, pilot studies with supplemental inosine (usually 1-2 grams daily) have yielded promising, though not yet definitive results in multiple sclerosis patients.[203-205] (In a more recent study, concurrent administration of inosine failed to improve

the response to interferon-beta therapy in MS patients.[206]) Dr. Ariel Reyes has proposed that the lower risks for Alzheimer's disease and vascular disorders noted in patients receiving diuretic therapy might reflect, at least in part, the fact that these drugs raise blood urate level by blocking kidney excretion of urate.[207] So supplemental inosine may have real clinical potential for preventing or treating peroxynitrite-mediated disorders, particularly those of the central nervous system.

But we mentioned that there are some drawbacks. The chief of these is that uric acid has limited solubility, with the consequence that, in people with very elevated blood and tissue levels of uric acid, uric acid crystals can precipitate out, inducing the painful clinical syndrome known as gout. Gouty arthritis can be extremely painful – though it is often readily corrected by suppressing the body's production of uric acid with drugs that inhibit xanthine dehydrogenase, such as allopurinol. More ominously, precipitation of uric acid crystals in the kidney tubules (gouty nephropathy) sometimes leads to life-threatening kidney failure. This latter complication can often be avoided by consuming ample amounts of fluid and maintaining an alkaline urine, as urate is relatively soluble under alkaline conditions. Nonetheless, 4 of 16 MS patients treated with inosine developed renal stones in a recent trial.[205] Clearly, supplemental inosine should be used under a physician's supervision, with regular monitoring of blood urate levels; an alkaline diet (moderate in protein, high in potassium-rich fruits and vegetables) and high fluid intake should help minimize risk for gouty nephropathy. In those people whose uric acid levels are near the upper limit of normal, the safe dose may be zero!

A further problem with employing urate as a peroxynitrite scavenger is that there is limited scope for enhancing its scavenging efficacy. Even in people with relatively low baseline uric acid levels, it won't be safely feasible to increase tissue or brain levels more than about two-fold. That means that much of the peroxynitrite-scavenging potential or urate is already actualized in normal healthy people. (In contrast, it is possible to enhance intracellular levels of reduced folates in many tissues by tenfold or more.)

Finally, there is some concern that increased dietary purine levels may have countervailing effects on tissue oxidative stress. In pathological conditions in which xanthine dehydrogenase has been converted to xanthine oxidase in affected tissues, the conversion of purines to uric acid entails the concurrent production of superoxide. Furthermore, in rodents and many other species, uric acid is a toxin that promotes oxidative stress – apparently by activating NADPH oxidase! Indeed, these species have an enzyme – not found in primates such as humans – that converts uric acid to the compound allantoin, which is innocuous (but which won't scavenge peroxynitrite-derived radicals). Primates have evolved the ability to tolerate much higher concentrations of urate than many other species can. Recent studies with cultured human cells suggest that urate also has the capacity to activate NADPH oxidase in some human cells[208, 209] – but that this capacity is almost maximized by the urate concentrations found in people whose blood urate is at the lower edge of normal – in other words, raising urate levels in humans would have at most a very modest impact on NADPH oxidase activity. And urate might be

expected to sometimes have a countervailing favorable impact on superoxide production, by protecting NO synthase from peroxynitrite.

In epidemiological studies, high blood levels of urate tend to correlate with increased cardiovascular risk.[210] However, this might simply reflect the fact that urate levels tend to be elevated in insulin resistance syndrome, which is quite common and which increases vascular risk through various mechanisms that have nothing to do with uric acid.[211, 212] The possibility that uric acid might promote vascular disease has also been discounted by several clinical studies in which measures for either increasing or decreasing blood urate levels have had no apparent impact, at least in the short term, on vascular function.[213, 214] Moreover, raising blood urate levels by intravenous infusion was found to *improve* vascular function in smokers and type 1 diabetics[215] – a result which may reflect an important role for peroxynitrite in the arterial disease associated with these disorders. And inosine therapy does not raise blood pressure in MS patients.[216] Hence, current evidence suggests that moderate increases in blood urate levels do not boost vascular oxidative stress to a meaningful degree.

It is intriguing to note that, just as high-dose folate had a half-crazy but brilliant clinical advocate several decades ago, the same can be said for dietary nucleic acids. Dr. Benjamin Frank recommended dietary nucleic acids as an "anti-aging" strategy, writing several popular books in which he exalted the alleged benefits of dietary nucleic acids as well as a number of other natural metabolites.[217] Dr. Frank was in fact a remarkably creative pioneer of "orthomolecular medicine", though his clinical research was never more than anecdotal. One observation he made was that dietary nucleic acids were useful in patients with congestive heart failure – converting it from a "three pillow" disorder to a "one-to-two pillow" disorder. (People with congestive failure often need to use multiple pillows at bedtime so that they can breathe well enough to sleep.) In this regard, we should draw attention to recent evidence that peroxynitrite compromises the efficiency of heart function in congestive heart failure.[158, 218] Perhaps we should try high-dose folate too!

Allopurinol versus Xanthine Oxidase

You will recall from our discussion of Dr. Oster that his use of high-dose folic acid was prompted by his belief that this would inhibit the pro-oxidant enzyme xanthine oxidase – a presumption that proved faulty. Moreover, Oster believed that the xanthine oxidase threatening our vascular systems arose by absorption of xanthine oxidase from inadequately heated cow's milk – another view that hasn't stood the test of time.

However, it now known that, when our tissues are under severe oxidative stress, they often can generate their own xanthine oxidase. The enzyme xanthine dehydrogenase – which, ironically, functions to produce uric acid from nucleic acid precursors – can be converted by oxidative stress to an alternative form known as xanthine oxidase.[219] Unlike the "healthy" enzyme from which it arises, xanthine oxidase produces superoxide when it generates uric acid. And this can act as a multiplier mechanism for oxidative stress. When mitochondria and/or NADPH oxidase produce excessive amounts of superoxide, the resulting oxidative stress can promote the

conversion of xanthine dehydrogenase to xanthine oxidase – which then futher boosts oxidative stress. This feedforward mechanism is analogous to what happens with NO synthase when it becomes uncoupled by peroxynitrite – excess superoxide production gives rise to more superoxide production!

Back in 1970, Dr. Oster (and medical science) knew none of this – but he correctly guessed that vascular oxidative stress was a key cause of vascular disease, and that xanthine oxidase was a key source of that stress – he erred only in presuming that this xanthine oxidase originated in cow's milk, rather than the body's own tissues. So, as we have noted, his first approach to treating coronary disease was to try the newly approved gout drug allopurinol (a.k.a. Zyloprim), a well documented inhibitor of xanthine oxidase – and he reported that it worked in patients with coronary angina.[163] He only shifted his focus to folic acid because he wasn't sure about the long-term safety of a drug that at that point was relatively new. But Oster's report that allopurinol was useful in angina was almost wholly ignored, and apparently no one tried to replicate his experience with it.

Well, guess what? Forty years later, Dr. Allan Struthers and collegues at the University of Dundee published a clinical study in the eminent medical journal Lancet providing compelling evidence that high-dose allopurinol (600 mg daily) is an effective treatment for anginal pain.[220] This study followed on the heels of previous work from this group demonstrating that allopurinol treatment could substantially improve the function of vascular endothelium in patients with heart failure – an effect which they concluded reflected a major reduction in vascular oxidative stress.[221] These workers suspect that the beneficial impact of allopurinol therapy in angina is primarily attributable to a reduction of oxidative stress in the endothelial lining of the coronary arteries, which enables them to dilate more normally in response to a downstream oxygen deficit.[221] A similar mechanism likely accounts for the benefit of high-dose folate in angina. It is conceivable that some of allopurinol's antioxidant benefit derives from a direct oxidant-scavenging action[222] – indeed, it is tantalizingly similar to urate in structure, and no one has yet determined whether it might quench peroxynitrite-derived radicals.

Angina is unlikely to be the only clinical syndrome in which xanthine oxidase-induced oxidative stress plays a pathogenic role.[223] Hence, we can anticipate that allopurinol will ultimately prove to have adjuvant value in a range of oxidant-driven disorders in which xanthine oxidase becomes activated in afflicted tissues. Conceivably, an antioxidant effect contributes to allopurinol's documented clinical utility in the treatment of chronic kidney disease.[224, 225] Fortunately, despite Oster's qualms, allopurinol has proven to be a relatively well tolerated drug – and it is now inexpensive.

Antioxidant Enzyme Induction

The final major strategy employed in Full-Spectrum Antioxidant Therapy is to ingest nutraceuticals and foods that can boost cellular expression of antioxidant enzymes and of the key peptide antioxidant glutathione. These compounds fall into two chief categories: so-called "phase 2 inducers", and the hormone melatonin.

Our cells have an important mechanism that, in response to perceived chemical threats, boosts their expression of antioxidant enzymes and of enzymes that aid detoxification of mutagens. This is known as a phase 2 response.[226] Most cells contain a protein known as Nrf2 capable of interacting with special regions of DNA (known as "antioxidant response elements") in a way that greatly increases cellular expression of a wide range of enzymes that exert antioxidant activities, or that aid mutagen detoxification (thereby aiding prevention of cancer). However, Nrf2 is often kept away from DNA and out of the nucleus because it binds tightly to a cytoplasmic protein, Keap1; this binding also promotes the proteolytic destruction of Nrf2 in intracellular complexes known as proteasomes. Keap1 is also capable of binding to a number of chemicals that, because of their reactive chemical structures (electrophiles), have the potential to attack DNA and give rise to mutations. In addition, oxidative stress can alter the structure of Keap1.[227] Either sort of structural modification disrupts the binding between Keap1 and Nrf2, with the result that Nrf2 becomes free to migrate to the nucleus, interact with DNA, and boost the synthesis of enzymes that protect us from mutagens and oxidants. More recently, it has been demonstrated that oxidative stress and electrophiles can also acutely stimulate the synthesis of Nrf2 by boosting the efficiency with which its mRNA is translated.[228, 229] Hence, these agents can increase the synthesis of Nrf2, protect it from degradation, and promote its uptake by the nucleus. It is good news that our cells possess such a logical self-protective mechanism. And it is better news that there are a great many natural phytochemicals that are capable of interacting with Keap1 and stimulating Nrf2 synthesis (in their native forms, or after metabolism) and thereby triggering the phase 2 response – *but that are inherently non-toxic*. In effect, our cells perceive these compounds as dangerous – but they aren't, and they actually protect our cells by inducing increased expression of cellular antioxidants.

Such phytochemicals are often found in foods suspected to reduce cancer risk; these foods include **cruciferous vegetables** (e.g. broccoli, cabbage, brussel sprouts, cauliflower, kale, etc.), **allium vegetables** (garlic and onions), **pomegranate**, and **green tea**. One of these compounds, **sulforaphane**, is now receiving considerable research attention; broccoli sprouts are particularly rich in it, and sulforaphane-enriched broccoli sprout extracts are being developed as nutraceuticals.[230, 231] The catechins in green tea (**EGCG**), and the sulfur compounds in garlic are also commonly studied. There is little doubt that a diet rich in these foods provides protection from oxidants and carcinogens, and their regular ingestion can be warmly recommended. However, a lot of clinical work is still required before extracts of these foods can be used as nutraceuticals with well-defined dose-dependent benefits as phase 2 inducers. Efforts are underway to turn sulforaphane into a nutraceutical, but this work is still in an early stage.

On the other hand, the antioxidant **alpha-lipoic acid**, which functions as a physiologically essential cofactor in bioenergy metabolism, but which also acts directly as a scavenging antioxidant when administered in supranutritional doses, has well documented clinical benefits in defined doses, and it now seems clear that these benefits are primarily reflective, not of its scavenging activity, but of its ability to activate the phase 2 response.[232-234] Lipoic acid has shown versatile neuroprotective activity in nearly two decades of rodent research, and it has

clearly been shown to be clinically beneficial in diabetic neuropathy, in daily oral doses of 600-1800 mg.[235, 236] Lipoic acid has also been used clinically in a range of liver disorders, including mushroom poisoning and hepatitis C.[237, 238] Although most scientists have thought of lipoic acid as an oxidant scavenger (the same way they thought of bilirubin), it has long been known that lipoic acid could increase cellular levels of glutathione, a crucial intracellular antioxidant. Only recently, it has been shown that this increase in glutathione reflects an increase in glutathione synthesis triggered by lipoate's phase 2 inductive activity.

This finally explains why lipoic acid is now showing such a wide range of protective effects in animal models of disorders characterized by excessive oxidative stress. In rodents, supplemental lipoate has shown anti-atherosclerotic activity, has helped to prevent diabetic complications, reduces ischemia-reperfusion damage, exerts anti-inflammatory effects, and, as noted, often protects neurons and the liver.[239-248] In pilot clinical studies, lipoic acid has shown a favorable effect on vascular function, and there are intriguing hints that it may be beneficial in the early stages of Alzheimers.[249, 250] Very likely, researchers are just now scratching the surface of lipoic acid's protective potential.

Because lipoic acid is readily available at affordable prices, and because there is such a large amount of pre-clinical and even clinical evidence documenting its versatile efficacy, it is a prudent choice for use as a phase 2 inducer in the context of Full-Spectrum Antioxidant Therapy. 1-3 600 mg capsules daily appear likely to be useful, consistent with its documented use in diabetic neuropathy. However, it is perfectly appropriate to employ additional phase 2-inducing phytochemicals (or foods providing such phytochemicals) in a comprehensive antioxidant regimen.

The antioxidant enzymes boosted by a phase 2 response include: heme oxygenase-1 (you'll remember its role in generating intracellular bilirubin), thioredoxin reductase, glutathione peroxidase, superoxide dismutase, catalase, and the enzyme that is rate-limiting for glutathione synthesis, gamma-glutamylcysteine synthetase. Also induced are a number of enzymes which participate in carcinogen detoxification.

The use of a phase 2 inducer like lipoic acid may be particularly appropriate in the context of other antioxidant measures such as PhyCB, since otherwise antioxidants are likely to *decrease* cellular expression of antioxidant enzymes by reducing the oxidative stimulus to phase 2 induction. In other words, an agent like lipoic acid can compensate for the missing oxidative stress, convincing cells that they are still at risk.

Green Tea Catechins

Green tea is particularly rich in a category of polyphenols known as catechins, members of the flavanol family. The chief of these, epigallocatechin gallate (EGCG), is well known for its potent phase 2 inducing activity.[251] Green tea tends to be richer in catechins than black tea because green tea is flash heated soon after harvesting; this destroys the activities of enzymes which otherwise promote the oxidation of catechins.

Tea is of course one of the world's most popular beverages, and in East Asia green tea is heavily consumed. This has made it feasible for epidemiologists to evaluate health outcomes in people who use green tea regularly. Recent reports in this regard have been remarkable and quite encouraging. In elderly subjects, heavy regular use of green tea has been linked to decreased total and cardiovascular mortality, as well as decreased risk for atherosclerosis, hypertension, cardiovascular events, strokes, dementia, depression, and osteoporosis.[252-264] Particularly noteworthy are recent findings derived from the Ohsake Cohort Study, which in 2006 enrolled about 14,000 reasonably healthy elderly subjects (65 or older) and assessed their lifestyle habits, including their consumption of green tea.[252] Over 3 years of follow-up, serious functional disabilities (as assessed objectively by the Japanese Ministry of Health, Education, and Welfare) developed in about 9% of these subjects, about 3% died. People who were consuming 5 or more cups of green tea daily at baseline, as compared to those consuming less than 1 cup daily, were found to be only about half as likely to develop functional disability during the follow-up period; those drinking intermediate amounts of tea achieved intermediate levels of protection. Since people who drank green tea heavily differed a bit in their dietary choices and social habits from those who didn't, the researchers did multiple regression analyses to attempt to negate the impact of these confounding factors on the analysis; after these adjustments, heavy consumption of green tea was still associated with a one-third reduction in risk for disability, and men and women appeared to benefit equally. The authors judged that the protection from disability enjoyed by the heavy green tea drinkers likely reflected reduced risk for stroke, dementia, depression, and fractures stemming from osteoporosis and muscle weakness. As noted, other studies have found that green tea drinkers are at lower risk for stroke, dementia, depression, and osteoporosis – and a recent controlled clinical study found that daily supplementation with green tea polyphenols (500 mg daily) improved muscle strength in postmenopausal women.[265] If these findings are replicable – they are certainly statistically robust in this study – they imply that frequent consumption of green tea (and likely green tea polyphenols) can have a tremendously positive impact on healthspan.

Green tea users appear to have decidedly superior cardiovascular health; risk for stroke in particular is notably diminished. Likely, this reflects a favorable antioxidant impact of green tea catechins on the arterial wall, mediated by phase 2 induction.[266] In controlled clinical studies, supplementation with mixed green tea catechins (580 mg daily) or pure EGCG (150 mg twice daily) in smokers or patients with coronary artery disease was shown to favorably influence endothelium-dependent vasodilation, suggesting that green tea catechins are capable to improving endothelial NO generation.[267-270] However, in the EGCG study, this benefit was seen only for several hours following EGCG ingestion, presumably reflecting the limited half-life (about 5 hours) of EGCG in the blood.[270, 271] Hence, people who wish to replicate the health protection associated with green tea consumption by taking supplemental green tea catechins, would seem well advised to take a number of modest doses of these catechins throughout the day, rather than taking one large daily dose.

The favorable impact of heavy green tea consumption on risk for cognitive dysfunction or dementia, may reflect in part a beneficial impact of green tea on cerebrovascular health.

However, rodent and cell culture studies suggest that EGCG, in low micromolar concentrations feasibly achievable via EGCG supplementation, can act directly on the brain to suppress production of the toxic amyloid beta protein thought to be driving force in the development of Alzheimer's disease.[272-276] EGCG appears to achieve this by promoting the protective alpha-secretase pathway whereby the amyloid precursor protein is metabolized.[272-274] Other studies show that EGCG can render neurons less sensitive to the toxic effects of amyloid beta.[277-281] How EGCG achieves this protection is not yet clear, although antioxidant mechanisms likely play a role. In any case, the potential of green tea catechins to prevent or postpone dementia is exciting, and strongly recommends their use in preventive health programs. While it seems likely that other antioxidants have important potential for dementia prevention[282, 283] – many of them show efficacy in rodent models of Alzheimer's disease – green tea can be considered of virtually proven benefit in this regard thanks to recent epidemiology.

Because of their phase 2 inductive activity, green tea catechins have shown cancer-preventive activity in carcinogen-treated animals.[284] Nonetheless, the impact of green tea consumption on cancer risk emerges as somewhat equivocal in epidemiological studies.[285] Some studies suggest that green tea users may be at lower risk for leukemias, lymphomas, and cancers of the cancers of the lung, colon, prostate, liver, endometrium, and ovaries; however, not all studies confirm these findings, and more evaluation is needed. Green tea has not appeared to influence breast cancer risk in prospective cohort studies, albeit case-control studies have sometimes reported protection.[286] While green tea may indeed reduce risk for certain malignancies, its influence on overall cancer mortality does not appear to be prominent[255, 264] – in contrast to its notable impact on cardiovascular mortality.

While only a small amount of epidemiology has associated green tea use with superior bone density, green tea catechins have proven protective in rodent models of osteoporosis.[264, 287] High daily intakes of green tea catechins have also modestly lowered body fat in some controlled clinical studies, likely reflecting an increase in the efficiency of fat oxidation.[288-292]

A typical Japanese cup of green tea is said to provide about 80 mg of catechins,[267] and intakes of 5 or more cups daily have been associated with marked health protection. Hence, a supplemental intake of at least 400 mg green tea catechins, spread throughout the day, may be a prudent strategy for those who are unwilling or unable to drink large amounts of green tea.

Melatonin as an Antioxidant

There is another key mechanism – complementary to the phase 2 response – that boosts the production of antioxidant ezymes throughout the body. The hormone melatonin, produced principally by the pineal gland at the base of the brain, induces antioxidant enzymes in a range of tissues, presumably by interacting with special nuclear receptors; the range of enzymes induced is very similar to that evoked by phase 2 responses.[293] Melatonin also regulates cellular functions by stimulating melatonin receptors on the surface of cells. Melatonin also can act as a direct scavenger of many sorts of radicals,[294] though it is likely that in the moderate doses employed clinically its major antioxidant activity is indirect, via enzyme induction. Melatonin

is a small molecule that is efficiently absorbed intact, and, unlike many hormones, it is readily available in the U.S. as a non-prescription nutraceutical.

There is a major nocturnal bump in pineal production of melatonin, and this is thought to play a role in regulating the body's day-night (circadian) physiological rhythms. Melatonin also boosts immune functions by stimulating the activity of so-called "antigen-presenting" immune cells that provide activating signals to other immune cells that have cancer scavenging activity – cytotoxic T lymphocytes and natural killer cells.[295, 296] There is compelling clinical evidence from Milanese cancer researchers that nocturnal melatonin supplementation prolongs survival in cancer patients while mitigating some of the side effects of chemotherapy.[297-300] Nocturnal melatonin production tends to fall off as people get older, and it is speculated that this contributes to the increased risk of cancer experienced by elderly people[301] (though gradual lifelong accumulation of mutations in stem cells is probably the major factor in this regard).

Not surprisingly, melatonin has shown protective activity in many rodent models of human disease – ischemia-reperfusion damage associated with stroke, rodent models of Alzheimer's and Parkinson's diseases, traumatic brain and spinal cord injury, complications of diabetes, and drug toxicities.[302-308] Much of the available research focuses on neurological problems, as neurophysiologists are particularly interested in melatonin. Dr. Russell Reiter of the University of Texas, San Antonio, one of the foremost melatonin researchers, has been astoundingly prolific in proposing potential clinical applications for this molecule.

Since melatonin production tends to fall off with increasing age, we particularly recommend nocturnal melatonin supplementation for elderly people – 5-10 mg at bedtime. It may be important to give melatonin only at night, since in this way the natural biorhythms which melatonin promotes are reinforced rather than antagonized.

N-Acetylcysteine Boosts Glutathione

A key way in which both phase 2 inducers and melatonin enhance cellular antioxidant defenses is by increasing the production of an enzyme, gamma-glutamylcysteine synthase, that is rate-limiting for synthesis of the intracellular oxidant scavenger glutathione.[309-312] Glutathione, which is found in relatively high (millimolar) concentrations in most cells, functions directly as an efficient and versatile oxidant scavenger, but it also works with various enzymes (such as glutathione peroxidase and glutaredoxin) to prevent and reverse oxidative damage, antagonizes the pro-inflammatory effect of hydrogen peroxide on cell metabolism, and plays a key role in the detoxification of carcinogens and other toxic molecules.[313-319] Glutathione is distributed throughout the cell, and, along with CoQ, is the chief scavenging antioxidant in mitochondria. Glutathione is synthesized from 3 amino acids; one of these, cysteine, is usually present in relatively low concentrations within cells; thus, its availability can determine the rate at which glutathione in produced. Dietary protein is a good source of cysteine, but its availability can also be enhanced via supplementation. However, cysteine supplements per se are not very useful for this purpose, because in concentrated form cysteine can produce severe gastrointestinal irritation, and is rather poorly absorbed. In contrast, its natural derivative N-

acetylcysteine (NAC) is well absorbed, well tolerated, and readily enters the body's cells. Once inside cells, the acetyl group is cleaved off to generate cysteine, which can then be used in the synthesis of glutathione as well as proteins.[320] NAC supplementation has been shown to boost cellular and blood levels of glutathione, both in rodents and humans.[320-322] Moreover, NAC in the bloodstream and in the spaces between cells has direct antioxidant activity, and can react direct with mucus proteins to reduce their viscosity.[323] Hence, NAC is often used to treat inflammatory lung disorders characterized by excessive mucus production; indeed, it was originally introduced in the 1960s as a "mucolytic" agent – and only later discovered to boost glutathione levels.

NAC is available both as a nutraceutical and as a drug. To date, physicians have used NAC primarily to protect the liver from chemicals that are hepatotoxic, and in the management of chronic inflammatory lung disorders, typically in oral doses of 600-1800 mg daily that are convenient and well tolerated.[324-328] Its utility for these applications in well documented. However, rodent studies and, to a more limited extent, clinical trials, have shown that NAC has potential for preventing or treating a wide range of disorders in which oxidative stress plays a key pathogenic role: atherosclerosis, diabetic complications, neurodegenerative disorders, certain psychiatric conditions, and HIV, among others.[320, 329-338] One particularly intriguing controlled clinical study found that, in elderly subjects, NAC supplementation during the winter months markedly reduced symptoms related to influenza; although it didn't prevent the initial infection, NAC users were far less likely to experience annoying symptoms – two-thirds less likely![339] (The fact that, in the subsequent decade, no published controlled studies have attempted to replicate this highly promising finding, is a rather damning indictment of the role that big money plays in clinical medical research; no one can get rich from NAC!) Rodent studies likewise demonstrate that NAC can confer protection from the flu.[340-342] This protection presumably reflects a key role for oxidative stress in the inflammatory response evoked by influenza infection.[343] (Killer flus kill not because of the direct cytotoxicity of the flu virus, but rather because of the over-exuberant inflammatory response they can evoke – the lungs literally fill up with fluid and inflammatory cells, impairing respiration.)

Phase 2 inducers, and/or melatonin, can be expected to work hand-in-glove with NAC to boost cellular glutathione levels. Indeed, a complementary impact of co-administered lipoic acid and NAC on oxidative stress in cells cultured from Alzheimer's patients has been reported.[344] We recommend an NAC intake of 600 mg, twice daily, which is convenient, well tolerated, affordable, and within the range typically used to treat chronic lung disorders. (This is also the dosage schedule shown to prevent flu symptoms.) Higher doses can be used in acute care situations, but can be associated with side effects such as nausea. Since the body metabolizes cysteine to yield sulfuric acid, which can adversely affect bone density, it may be smart to eat plenty of fruits and vegetables or use an organic potassium supplement when using NAC on a continuing basis. (See the discussion on potassium below.)

Another appropriate oral delivery form for cysteine is its disulfide, cystine; cystine is readily taken up by cells, where intracellular reduction converts it to free cysteine.[345] Cystine has received less research attention than NAC, possibly because the latter has direct antioxidant activity in cell culture studies. One of the few recent clinical studies employing cystine suggests that it can blunt certain immunosuppressive effects of exercise training.[346]

The Vitamin/Mineral Antioxidants

We have now described and explained the key components of a **Full-Spectrum Antioxidant Therapy**: reduction of NADPH oxidase activity with PhyCB from spirulina; protecting the structural and functional integrity of mitochondria and moderating their propensity to produce superoxide with astaxanthin and (optionally) coenzyme Q10; scavenging of peroxynitrite-derived radicals with high-dose folate and (optionally) inosine; induction of antioxidant enzymes and promotion of glutathione synthesis with the phase 2 inducers lipoic acid and EGCG, nocturnal melatonin, supplemental NAC, and frequent ingestion of key protective foods such as cruciferous vegetables, garlic, onions, and green tea.

But it is also desirable to insure adequate intakes of essential micronutrients that fulfill antioxidant roles, such as selenium, and vitamins E and C. For those who may not consistently consume diets that are optimally nutritious, this can best be insured with "nutritional insurance supplementation" – a term devised by the great nutritionist Dr. Roger J. Williams to refer to comprehensive vitamin-mineral supplements that insure complete micronutrient nutrition.[347, 348]

One of the most intriguing of the essential micronutrient antioxidants is the trace mineral **selenium**, which is a key component of a range of antioxidant ezymes, including thioredoxin reductase and the several forms of glutathione peroxidase.[349] Glutathione peroxidase eliminates hydrogen peroxide (like the enzyme catalase), as well as organic peroxides generated from membrane fats; thioredoxin reductase helps to reverse the oxidative effects of hydrogen peroxide on proteins. Collectively, the selenium-dependent antioxidant enzymes reverse the pro-inflammatory effects of hydrogen peroxide signaling in cells.

European epidemiological studies strongly suggest that selenium status has a very meaningful impact on cardiovascular risk – with lower risk seen in those with relatively high selenium levels – whereas American studies general fail to observe this.[350, 351] This may simply reflect the fact that soils in many parts of Europe tend to be relatively low in selenium, while the American Midwest, where most American grain is grown, tends to be comparatively selenium-rich. Thus, poor selenium status is much more common in Europe than America. Once the diet provides a certain minimal level of bioavailable selenium (around 70 micrograms daily), the body's capacity to utilize selenium for antioxidant enzyme production has been maximized, such that additional dietary selenium will not further boost antioxidant protection. This implies that supplemental selenium will aid the antioxidant defenses of people with relatively low baseline selenium status, but not those whose baseline status is relatively high.

Although there is ample reason to suspect that supplemental selenium might decrease cardiovascular risk in certain European populations with poor selenium nutrition, long-term supplementation trials to assess this have not yet been undertaken – with one recent exception. In a 5 year study, 443 Swedish subjects over 70 received either a combination of selenium and coenzyme Q10, or a placebo. Over the five years, cardiovascular mortality was found to be about half as high in those receiving placebo.[352] These finding are quite provocative – though it is a shame that selenium *alone* wasn't also evaluated in this study; coenzyme Q10 is a relatively expensive nutraceutical, whereas nutritional doses of selenium are dirt cheap, and could readily be employed for population-wide prevention.

Studies in carcinogen-treated rodents reveal that selenium, in slightly supranutritional levels, has an anticarcinogenic potential that is unrelated to selenium's nutritionally essential antioxidant role, and seems to be mediated by the selenium metabolite methylselenol.[353-355] This phenomenon might reflect increased cell suicide ("apoptosis") in cells that have been damaged by mutagens, and that are therefore pre-cancerous, and/or a strengthening of DNA repair mechanisms.[356-358] Moreover, some epidemiological studies have correlated higher selenium status with lower risk for certain cancers. These findings motivated a landmark supplementation study organized by Dr. Larry Clark. When over 1300 Americans with a previous history of skin cancer took either a selenium supplement (200 mcg selenium in brewer's yeast) or a matching placebo for over ten years, significant reductions in risk for prostate, colon, and lung cancer were observed in those taking selenium – and a halving of the cancer death rate![359] Closer analysis of the data, however, revealed that the reduction in cancer incidence was confined to those with lower baseline selenium levels – with very substantial protection (a halving of total cancer incidence) in the bottom third of the distribution (serum selenium less than 105 mcg/l.)[360-362] And a further smaller study, in which 400 mcg of selenium daily was given, failed to show an impact on cancer risk.[363] More recently, a large follow-up study, the SELECT trial,[364] did *not* achieve a reduction of prostate cancer incidence with 200 mcg of selenium daily (albeit this study employed chemically-synthesized selenomethionine rather than yeast selenium); whether baseline selenium status influenced response in this study is not yet clear, as the full data aren't yet published. While Clark had taken care to target a population in the American southeast where selenium status was relatively poor, the average baseline selenium level in the SELECT study was 137 mcg/ml. Notably, in Clark's study, those with baseline selenium above 121 mcg/l (the upper third of the distribution) achieved no cancer protection from supplemental selenium – so his findings were not necessarily inconsistent with those of the SELECT study.

Overall, the data suggest that, as in the case of cardiovascular disease, the benefits of supplemental selenium for human cancer prevention may be largely confined to people with relatively poor selenium status, and that modest doses of selenium may be sufficient to achieve this benefit.[362] A recent meta-analysis, lumping together the results of 9 controlled selenium supplementation trials, many from China, yields results consistent with this conclusion – selenium supplementation reduced cancer incidence by about a third in low-selenium populations.[365] But further research will be required to validate this view, preferably targeting

low-selenium populations. In any case, the failure of high-dose selenium to lessen human cancer incidence serves to underline the fact that rodents treated with sudden large doses of carcinogens are poor models for spontaneous cancer incidence in humans, in whom mutations usually accumulate gradually.

It is entirely reasonable to suspect that adequate selenium status could provide protection from cancer. The selenium-dependent enzyme glutathione peroxidase helps to control cellular levels of hydrogen peroxide, which can induce mutagenic damage in DNA when it interacts with free iron (as discussed below).[366] Therefore, selenium may function physiologically to lessen inflammatory mutagenesis.

Although the essential mineral **zinc** plays a number of essential roles in the body – poor zinc status can compromise immune defenses and wound healing , for example – good zinc status provides protection from oxidative stress for reasons that remain rather obscure.[367] Zinc is an essential cofactor for the cytoplasmic form of superoxide dismutase, so severe zinc deficiency can compromise this activity. Increased intakes of zinc boost expression of the protein metallothionein, which binds to zinc and copper via its numerous sulfhydryl groups, acting as a storage reservoir for these minerals.[368-370] These sulfhydryl groups can also function as radical scavengers, and hence metallothionein induction may mediate some of the antioxidant protection afforded by zinc. Moreover, metallothionein protects tissues from the toxic impact of the heavy metal contaminant cadmium by binding tightly to it, preventing it from interacting with other targets in cells.[371, 372] Recent epidemiological research links above-average body stores of cadmium to increased risk for vascular disease, cancer, diabetes, osteoporosis, and kidney dysfunction, and studies in rodents and cell cultures give us reason to suspect that cadmium may play a mediating role in these disorders.[373-376] Indeed, one of the chief mechanisms whereby cadmium promotes pathology is by inducing oxidative stress – which may help to explain why it has been linked to such a wide range of disorders.[377] These considerations imply that supplemental zinc, or a zinc-rich diet, has the potential to antagonize the toxic and pro-oxidant effects of cadmium by boosting the expression of cadmium's natural antagonist metallothionein. This view is borne out in rodent studies in which zinc administration has provided protection from cadmium's toxicity.[378-380] If further research provides confirmation that cadmium contamination is a major driver of human pathology, zinc supplementation may gain prominence as a feasible way of mitigating this damage. (Cadmium's toxicity might also be opposed by other effective antioxidant measures – note that spirulina can prevent cadmium's teratogenic effects in mice[381] – and by suppressing the absorption of dietary cadmium by ingesting ample amounts of essential minerals.[382-387])

Zinc may have been primarily responsible for the benefit observed in one of the few major clinical trials of antioxidant supplementation to have achieved a successful outcome: the AREDS1 study, which demonstrated that a supplement containing vitamin C, vitamin E, beta carotene, and zinc (80 mg daily) could slow the progression of advanced age-related macular degeneration, the leading cause of partial blindness in the elderly.[388] Moreover, those fortunate enough to be randomized to receive zinc in this study were 27% less likely to die during 6.5

years of follow up[389] (likely in part owing to zinc's immunosupportive and anti-inflammatory activities[390, 391]). Another recent study found that, in elderly subjects, supplementation with 45 mg of zinc daily led to significant reductions in markers of systemic inflammation such as C-reactive protein that correlate with increased cardiovascular risk. [373]

Zinc intakes that are excessively high can decrease blood levels of protective high-density lipoprotein, and can also antagonize copper absorption.[392, 393] For this reason, it may be wise to keep supplemental zinc intakes below 100 mg daily, and to include a small dose of copper when moderately high zinc doses are used. (The AREDS1 study included 2 mg copper when supplementing with 80 mg zinc.)

Another essential antioxidant that has received considerable evaluation is **vitamin E –** a.k.a. alpha-tocopherol. This functions to protect the fats in cellular membranes from oxidative damage. Substantial oxidant damage to membranes can cause cell death and dysfunction, which is why at least a minimal intake of vitamin E is essential in rodents. However, whether modulating vitamin E levels within the "adequate" range can have much impact on human health is very much in doubt. Vitamin E status does not influence cellular hydrogen peroxide levels, and thus doesn't have a clear impact on cell signaling mechanisms. (You will recall the analogy – scavenging antioxidants are like mops that only act on *part* of the floor.) Theoretically, vitamin E could influence signaling by decreasing production of the compound 4-hydroxy-2-nonenal (4-HNE), which can react spontaneously with proteins, interfering with their function. But a controlled clinical study in which supplemental dose of vitamin E was administered in a range of doses (up to 2000 IU daily) failed to observed any effect of this supplementation on urine levels of 4-HNE, or on blood levels of another marker for oxidative stress.[394] It was hoped and expected that vitamin E supplementation would decrease cardiovascular risk by decreasing oxidative damage to LDL particles[395] (which are cholesterol rich, are thought to mediate the adverse effect of high blood cholesterol on vascular health, and appear to be more toxic when oxidized), but extensive supplementation trials have failed to confirm this benefit.[396, 397] These findings suggest that, contrary to previous speculation, peroxidative damage to LDL does not play an obligate role in the atherogenic process (which does not rule out the possibility that other types of LDL structural modification, not inhibitable with vitamin E, do). Indeed, there was even a hint in some of these studies that vitamin E was modestly increasing risk;[398] some scientists speculate that this reflects an adverse effect of high supplemental intakes of vitamin E (a.k.a. alpha-tocopherol) on the nutritional availability of **gamma-tocopherol**, a related dietary compound that has the potential to scavenge peroxynitrite in cell membranes.[399-401]

Alpha-tocopherol is only one member of a group of structurally-related antioxidant compounds found in natural foods known as tocopherols – of which gamma-tocopherol is usually the most prominent. Nutraceuticals supplying the full range of natural tocopherols are known as "mixed tocopherols". Alpha-tocopherol gained its status as a vitamin because diets devoid of it caused sterility in rodents, whereas omitting other tocopherols from rodent diets didn't cause evident harm. But the laboratory of Dr. Bruce Ames made the intriguing discovery that, unlike alpha-

tocopherol, gamma-tocopherol could protect membranes by scavenging peroxynitrite-derived radicals – an effect associated with potent anti-inflammatory effects in rodents.[402, 403] Subsequent research showed that a high intake of alpha-tocopherol could impede the delivery of gamma-tocopherol to the body's tissues by monopolizing a key blood transport protein.[404] These findings raised the possibility that too high an intake of pure alpha-tocopherol might have a countervailing adverse impact on anti-inflammatory defenses by impairing the protective activity of gamma-tocopherol.[399, 405]

The results of the recent SELECT study indicate that these concerns may well be warranted; moreover, these results may be interpreted as evidence that dietary gamma-tocopherol functions physiologically to lessen cancer risk. Researchers were shocked to find that subjects randomized to receive 400 IU alpha-tocopherol daily subsequently were experiencing a statistically significant 17% increase in risk for new prostate cancer – a finding which led to early termination of the study.[406] In this regard, oral administration of mixed tocopherols rich in gamma-tocopherol has been shown to decrease prostate cancer occurrence in TRAMP mice, bioengineered to experience a high "spontaneous" incidence of this cancer.[407] An analogous effect has been reported in a rat strain genetically prone to this cancer.[408] Other studies show that gamma-tocopherol can exert growth-retardant effects on prostate cancer cells in culture.[409-412] Moreover, several epidemiological studies have found that increased dietary intakes or tissue levels of gamma-tocopherol correlate with decreased risk for prostate cancer in humans.[413-416] Arguably, the disappointing findings of the SELECT study may reflect the fact that high-normal dietary intakes of gamma-tocopherol provide meaningful protection from prostate cancer, but that this protection can be abrogated by supplementation with high doses of alpha-tocopherol. A follow up to the SELECT study, evaluating mixed tocopherols rich in gamma-tocopherol, rather than pure alpha-tocopherol, would appear to be warranted. In any case, in light of present evidence, those choosing to supplement with "vitamin E" might be well advised to use mixed tocopherols rather than a pure alpha-tocopherol preparation. Evaluation of the clinical potential of supplementation with either gamma-tocopherol or mixed tocopherol preparations is still in a early stage,[417, 418] though, as noted, intriguing anti-inflammatory effects in rodent studies – not achievable with alpha-tocopherol - have been reported.[402, 403, 419, 420]

It is now abundantly clear that supplemental vitamin E is not a "wonder nutrient" - probably in part reflecting the fact that people who get at least a modest amount of plant oils in their diets are likely to have adequate vitamin E status. Rodents appear to be more responsive to high intakes of vitamin E than are humans. The one area in which supplementation with high-dose vitamin E has shown some consistent clinical merit is in the treatment of non-alcoholic steatohepatitis, where it shows anti-inflammatory benefit.[421-423] But even here, mixed tocopherols might prove to be more protective.

Vitamin C is another antioxidant micronutrient that has received considerable clinical scrutiny. Vitamin C's essentiality reflects its role in collagen synthesis, but it can function as a versatile water-soluble radical scavenger, and its concentration in some tissues is similarly to that of glutathione. In particular, it acts to "re-arm" certain other antioxidants, such as vitamin E, after

they have lost an electron while quenching a radical. Vitamin C can restore the proper function of tetrahydrobiopterin (the key cofactor of NO synthase) after it has been attacked by peroxynitrite-derived radicals.[424] Unfortunately, supplemental vitamin C – like vitamin E – may have limited scope for aiding antioxidant defenses in people whose diets include reasonable amounts of fruits and vegetables. Once the diet provides a certain minimal amount of vitamin C – around 500 mg per day – the capacity of vascular cells to take up vitamin C from the blood has been saturated, so that intracellular levels do not increase further.[425-427] (Remember that, by like reasoning, we don't think that high-dose folic acid will improve antioxidant defenses in the brain.) So it is not likely that vitamin C supplementation trials, at least in relatively well-off populations, will show much impact on antioxidant defenses or health – and this seems to be borne out by most of the evidence to-date.[397] Nonetheless, it is clearly worthwhile to assure adequate vitamin C status (just ask anyone who has suffered from scurvy!), and the possibility that supplemental vitamin C might modestly reduce the duration of colds seems to be supported by an overview of current evidence.[428]

Whereas oral vitamin C seems unlikely to have much impact on the intracellular ascorbate content of most tissues in people with decent baseline nutrition, it is feasible to maintain somewhat elevated *serum* levels of ascorbate if high doses are given every few hours. Conceivably, some of the clinical claims for high-dose oral ascorbate regimens may reflect this fact.

Sooner or later you are likely to hear a medical "expert" opine that antioxidant supplementation has little to offer for health – based on disappointing results in controlled trials with vitamins E and C; he may even suggest that perhaps oxidants are good for us! You need to understand that the chief reason why these trials have shown little benefit is that they have had little impact on oxidant control. In contrast, the core strategies that comprise Full Spectrum Antioxidant Therapy are likely to have a substantial impact – at least in people threatened with excess oxidative stress.

Whereas oral administration of high-dose vitamin C may have limited utility in prevention or therapy, injectible high-dose vitamin C may have intriguing potential in clinical medicine. Very-high-dose infusions of sodium ascorbate can actually act as a pro-oxidant, generating superoxide and hydrogen peroxide in the spaces between cells; this comes about owing to iron-catalyzed transfer of an electron from ascorbate to molecular oxygen.[429] Normal cells have sufficient antioxidant activity to cope with the hydrogen peroxide produced under these circumstances. However, since cancer cells are frequently deficient in antioxidant activity – in particular, many are deficient in the enzyme catalase that disposes of hydrogen peroxide – they may be selectively susceptible to being killed or damaged by ascorbate-catalyzed oxidative stress.[430-432] Moreover, many cancers make increased amounts of superoxide, which can interact with ascorbate-generated hydrogen peroxide to produce deadly hydroxyl radicals.[433] High-dose intravenous sodium ascorbate is now being seriously studied as a strategy for shrinking cancerous tumors, without harm to healthy tissues. This strategy may also have the

potential to amplify response to concurrent administration of chemotherapy drugs whose killing mechanism is contingent on or potentiated by oxidant stress.[434, 435]

On the other hand, the oxidized form of vitamin C – dehydroascorbic acid (DHA) – may have clinically important *anti*oxidant activity when infused intravenously. Unlike ascorbic acid, DHA is avidly taken up by cells, via transport proteins whose chief function is to enable glucose uptake.[436] Once inside cells, DHA is rapidly converted (reduced) to ascorbate, which effectively becomes trapped inside the cell. Thus, high-dose infusions of DHA can be used to achieve marked increases in the intracellular ascorbate content of cells – whereas infusions of ascorbate per se would not achieve this aim. Since ascorbate is a versatile scavenger of free radicals, this strategy may have a clinically important antioxidant effect in emergency medical conditions associated with dangerous levels of oxidant stress.[437-439] Such conditions include reperfusion damage following a heart attack or a stroke, as well as septic or hemorrhagic shock, or severe burn injury. Infusion of DHA has been shown to be markedly protective in mouse models of stroke, even if infused after a cerebral artery is temporarily or permanently occluded.[437, 439] Arguably intravenous antioxidant therapy for heart attack, stroke, and various shock conditions could also incorporate PhyCB, leucovorin, as well as the natural metabolite pyruvate (or its more stable derivative ethyl pyruvate); in sufficient concentrations, pyruvate has the remarkable ability to promote the harmless destruction of hydrogen peroxide.[440-442] Unfortunately, leucovorin is the only one of these agents currently approved for intravenous administration. Oral administration of DHA may have little utility, since it will be converted to ascorbate in intestinal cells before it can reach the circulation.

Carotenoids Afford Protection from Light-Generated Oxidants

Carotenoids are fat-soluble compounds found in many fruits and vegetables that have scavenging antioxidant activity. We have discussed the outstanding health protective potential of astaxanthin, a so-called oxygenated or "xanthophyll" carotenoid. However, AX is a negligible component of most natural human diets, whereas other carotenoids are found in physiologically meaningful amounts in fruits and vegetables. **Beta-carotene** is often considered nutritionally essential, as it can be converted in the body to vitamin A (retinol), which has essential functions in vision and immune defenses. Unless you eat foods like liver or fish oil that contain pre-formed retinol, you will need dietary beta-carotene to make your own vitamin A. Moreover, dietary beta carotene is superior to vitamin A from a safety standpoint, since your body will only make as much vitamin A as you need when you ingest beta carotene. There is recent evidence that intakes of pre-formed vitamin A in only slight excess of the RDA can have a long-term adverse effect on bone density and fracture risk[443, 444] – whereas this problem hasn't been seen with even high intakes of beta-carotene.

Although carotenoids can scavenge many types of radicals, they are particularly distinguished by the fact that they can detoxify an oxidant known as singlet oxygen. This oxidant is produced when UV-A light interacts with certain photosensitizing chemicals in light-exposed tissues; the photosensitizers absorb the light energy and pass it on to molecular oxygen, which becomes

unstable singlet oxygen. Singlet oxygen reacts spontaneously with unsaturated fats and certain of the amino acids found in proteins, damaging their structures.[445] The pro-inflammatory effects of UV-A light on skin cells (keratinocytes) are mediated by singlet oxygen, which somehow induces the persistent activation of NADPH oxidase; in this way, transient exposure to UV can lead to longer-term inflammation.[446] Singlet oxygen is generated in the delicate retina of the eye by UV exposure; this presumably is why the macula, the part of the retina that receives most intense light exposure, contains high concentrations of the carotenoids **lutein** and **zeaxanthin** – the so-called macular pigment. Persistent oxidative stress in the macula appears to be a key factor in the induction of macular degeneration, the most common cause of blindness in the elderly; it is thus intriguing that diets rich in lutein/zeaxanthin have been linked to decreased risk for this disorder.[447, 448] In particular, dietary spinach, a rich source of lutein, emerges as protective in this regard. We should note that spirulina, of exceptional interest because of it rich store of PhyCB, is also a rich source of carotenoids – most notably zeaxanthin.

Epidemiological studies suggest that carotenoid-rich diets may reduce risk for certain types of cancer; in particular, there has been interest in the possibility that the carotenoid **lycopene** may reduce prostate cancer risk, and pilot supplementation trials with lycopene-rich tomato extracts in patients with early prostate cancer have yielded modestly encouraging results.[449-451] A favorable impact of lycopene supplementation on the progression of benign prostate enlargement has also been reported recently.[452] There is suggestive evidence that lycopene might reduce risk for cancer and other proliferative disorders in some tissues by increasing their production of a protein that antagonizes the activity of the key cancer promoting hormone IGF-I.[453-455]

Nonetheless, the data linking increased carotenoid intakes to reduced cancer risks are fairly inconsistent, and a mechanistic basis for carotenoid-mediated cancer prevention hasn't yet been clearly established, so no clear conclusions are possible at this time.[456-460] Surprisingly, a controlled clinical study concluded that high supplemental intakes of beta-carotene caused a modest *increase* of lung cancer risk in current smokers – most notably those who also consumed large amounts of alcohol.[461] No such effect was reported in past smokers or non-smokers. Studies in ferrets suggest that oxidants in cigarette smoke can convert beta-carotene to metabolites that interfere with vitamin A metabolism in the lung;[462] deficient vitamin A function has a cancer-promoting effect in smokers. (This finding is certainly ironic, in light of the fact that beta-carotene is the dietary precursor for vitamin A!) But more recent studies show that concurrent administration of other antioxidants, by preventing oxidative damage to beta-carotene, prevents the loss of vitamin A activity; indeed, this joint supplementation appeared to *reduce* risk for lung cancer in ferrets.[463, 464] So perhaps beta-carotene will indeed have anti-carcinogenic potential if used in the context of other effective antioxidant measures. (In this regard, it is interesting that Japanese smokers who carry the low-expression form of the HO-1 gene are more prone to lung cancer – suggesting a possible role for bilirubin and PhyCB in lung cancer prevention.[465] This gibes well with a report that lung cancer risk is lower in people with relatively high serum bilirubin.[20] Moreover, there is evidence that peroxynitrite is a mediator of

the DNA damage induced by lung cancer tar.[466] So it is conceivable that antioxidant prevention of lung cancer will indeed become feasible – once we employ the right antioxidants.)

In light of the quasi-essential role of lutein/zeaxanthin in retinal health, the essential role of beta-carotene as a vitamin A precursor, and the possibility that lycopene may provide protection from certain cancers, it seems reasonable to include at least modest doses of these carotenoids in nutritional insurance formulas.

Polyphenols Confer Vascular Protection

Plant-based foods, particularly fruits, vegetables, wines and teas, contain a vast range of polyphenolic compound that have direct oxidant scavenging activity. Epidemiological studies conclude that diets rich in these compounds are associated with superior vascular health. Although the health benefits linked to polyphenol-rich diets are often attributed to the scavenging antioxidant activities of these compounds, it seems likely that other effects make a more decisive contribution in this regard. As we have seen, some polyphenols, such as the catechins in green tea, have phase 2 inducing activity. But another important way in which some of these compounds can protect us is by triggering increasing vascular nitric oxide production.

The **flavanols** provided by raw **cocoa powder** may have particularly outstanding potential for health promotion. In particular, a prominent flavanol in cocoa powder, **epicatechin**, can act directly on the vascular wall to provoke increased production of protective NO.[467] Since NO has vasodilatory activity, this appears to explain why, in recent clinical studies, supplementation with cocoa flavanols has been shown to boost blood flow to the brain and skin (the latter effect improves skin moisture and appearance), decrease blood pressure in people with hypertension, and improve insulin sensitivity (possibly a reflection of increased delivery of glucose to muscle fibers).[468-473] French researchers have recently reported that dietary supplementation of rats with cocoa flavanols, beginning in middle age, blunts the age-related decline in cognitive performance and increases average lifespan.[474] Arguably, improved brain blood flow might contribute to this impact on cognitive function. If a similar effect could be achieved in humans, the implications would be immense!

Much of the excitement regarding cocoa flavanols stems from Harvard research focusing on the Kuna Indians, whose ancestral home is the Kuna Islands off the western coast of Panama. As long as the Kuna Indians live their traditional lifestyle on the Kuna Islands, they do not appear to develop hypertension, and stroke is extremely rare.[475, 476] There are a few other societies in which hypertension does not develop, but, in all of these societies, food is not salted. But remarkably, the Kuna Indians use as much salt as we do. What confers their remarkable protection from high blood pressure? The most interesting and novel aspect of the traditional Kuna diet is that, throughout life, they consume several servings of raw cocoa a day.[477] In light of the protective vascular effects of cocoa-derived epicatchin, this is the most likely explanation for their freedom from hypertension. It would be of great interest to evaluate the cognitive function of aging Kuna Indians who have maintained their traditional lifestyle.

Although dark chocolate has been touted as a rich source of cocoa flavanols, in point of fact most commercially available dark chocolate is a poor source of these protective factors, as the processing methods used for bulk production of cocoa destroy most of the flavanol activity. (Fortunately the more "primitive" methods used by the Kunas don't have this problem!) The Mars company has developed strategies for preserving the native flavanols in cocoa powder, and is now introducing consumer products featuring these flavanols.

Yet a recent study shows that, mg per mg, the widely distributed phytochemical **quercetin** may be more effective than epicatechin for provoking an acute release of NO from the cells that line our arteries.[478] Quercetin is found in apples, onions, red wine, tea, and a wide range of other foods; it is also available in crystalline form as a nutritional supplement. It is the most prominent member of a class of phytochemicals known as **flavonols**, constituting about two-thirds of the flavonol content of average diets.[479] (Note that epicatechin is a member of the flavanols, a related but structurally distinct set of compounds.) A number of epidemiological studies have examined correlations between total dietary flavonol intake and risk for heart attack or stroke; recent meta-analyses have concluded that people whose dietary intake of flavonols is in the upper third of the distribution, as compared to people whose flavonol intakes are in the lower third, are about 20% less likely to experience stroke or a fatal heart attack.[480, 481] Most of the analyzed studies use multiple regression strategies to rule out the possibility that high fruit and vegetable intake per se is responsible for this protection.

How does quercetin trigger endothelial NO release? Several studies suggest that quercetin may do this by opening potassium channels in the endothelial membrane.[482, 483] This results in an increase in the electrical charge across the endothelial membrane, which in turn boosts the rate at which calcium streams into endothelial cells. It is this extra calcium that triggers the increased production of NO by the enzyme NO synthase. This effect can be achieved with physiological relevant concentrations of quercetin, so it is a credible candidate for quercetin's true mechanism of action within the body. However, the bulk of quercetin in the bloodstream is present as certain metabolites, and it hasn't yet been shown that these metabolites can exert a similar action. If this is indeed the way that quercetin provokes NO release, the close structural homology between quercetin and epicatechin suggests that the latter may work by a comparable mechanism. Other cell culture studies point to additional mechanisms (activation of PI3K-Akt) whereby flavonoids may activate NO synthase, but whether these findings have relevance *in vivo* remains unclear.

Quercetin and related flavonoids also have the potential to modestly inhibit NADPH oxidase activity in endothelial cells. Hyperpolarization of the endothelial membrane has this effect, as discussed below in the context of potassium's antioxidant activity. In addition, a high proportion of absorbed flavonoids are methoxylated, and the 3'-O-methyl metabolites of various flavonoids can inhibit NADPH oxidase in a manner analogous to the phytochemical apocynin, commonly employed in rodent studies for this purpose.[484] However, whether this effect can be meaningful with feasible intakes of quercetin is unclear, and there is little evidence that

quercetin has important systemic antioxidant activity. (Apocynin has little clinical potential because massive doses would be required.)

In numerous rodent models of hypertension, dietary quercetin lowers elevated blood pressure – without influencing the blood pressure of rodents whose blood pressure is in the normal range.[485] These findings are paralleled by a clinical study observing a significant reduction of elevated blood pressure in people supplemented with 730 mg of quercetin daily.[486] Quercetin has also shown protective effects in rodent models of atherosclerosis.[487, 488] But perhaps the most remarkable finding with quercetin is its ability to boost the production of mitochondria in skeletal muscle and other tissues.[489, 490] This likely reflects the fact that NO has a stimulatory effect on this process, as demonstrated in rodents.[491, 492] Since mitochondria generate ATP, the catalyst that drives muscle contraction, and in particular are required for the utilization of fat as metabolic fuel, it's not surprising that quercetin supplementation has been shown to enhance the endurance of mice in either forced or voluntary exercise.[489] Although quercetin has not been found to boost performance in elite athletes, it does seem to modestly increase endurance when supplemented at 500 mg twice daily in untrained subjects, and also enhances the maximal rate at which people can utilize oxygen during exercise.[490, 493] Potentially, the impact of quercetin on tissue mitochondria content could also be of some benefit for control of obesity and insulin resistance syndrome. People who are overweight and insulin resistant tend to have fewer mitochondria and a diminished capacity to burn fat; this can make it harder for them to exercise effectively, and also may be partly responsible for the excessive tissue fat levels that characterize obesity and cause insulin resistance.[494] Indeed, high intakes of quercetin tend to diminish weight gain and improve insulin sensitivity in a rodent model of obesity, the Zucker fatty rat.[495]

Finally, quercetin shares the ability of a structurally similar compound – resveratrol, also found in red wine – to enhance the activity of the enzyme Sirt1, which is thought to have aging-retardant potential, and also has anti-inflammatory properties.[489, 490, 496, 497] Rodent and clinical research with quercetin is likely to be a growth industry for some time to come! Since dietary intakes of only 30-40 mg of flavonols per day have been associated with a meaningful reduction in risk for heart attack and stroke, the protection afforded by more substantial supplemental intakes may be prove to be quite noteworthy.

While on the topic of polyphenols, it is worth commenting briefly on commercial exploitation of ORAC, a technique for determining the extent to which consumption of dietary scavenging antioxidants can boost the radical scavenging potential of serum. Distributors of antioxidant-rich beverages and functional foods often point to the impact of their products on ORAC values as a demonstration of their products' efficacy. In fact, it is not clear that an increase in the non-specific scavenging activity of serum has an important health impact, as it may correlate poorly with *intracellular* antioxidant activity; most dietary polyphenols circulate in conjugated forms which have poor intracellular uptake. Moreover, as we have noted, some of the key benefits of dietary polyphenols and other antioxidants are mediated by phenomena such as phase 2 induction or NO release, which are not provoked by radical scavenging activity. Hence, while a

high ORAC value presumably reflects reasonable absorbability of the administered polyphenols, it may be a poor guide to the net health impact of a product.

Boosting Nitric Oxide as a Complement to Antioxidant Measures

In light of the ability of certain flavonoids such as quercetin and epicatechin to provoke endothelial NO synthase activation and NO release, it is pertinent to note that strategies which simultaneously boost NO production – within the moderate physiological range associated with good eNOS activity – while effectively controlling oxidative stress, may have extraordinary potential for health promotion.[498] NO, in moderate physiological concentrations that act primarily but not exclusively via activation of soluble guanylate cyclase, works in various ways to promote vascular health, prevent dementia , and maintain bone density – effects which can contribute importantly to healthy aging.[499-503] Yet oxidative stress tends to compromise NO production and bioactivity; as noted above, superoxide reacts directly and rapidly with NO to yield the potent oxidant peroxnitrite. Oxidative stress can also impair the activity of NOS in a number of ways – by impeding the synthesis or damaging the structure of NOS' obligate cofactor tetrahydrobiopterin, and by boosting levels of NOS' competitive antagonist ADMA (asymmetric dimethylarginine).[159, 504-506] Loss of tetrahydrobiopterin can be doubly damaging, as it results in an uncoupling of NOS such that it generates superoxide rather than NO; in this way, oxidative stress begets more oxidative stress![159] Indeed, in the context of oxidative stress, activating or increasing the expression of NO synthase may be counterproductive. Hence, concurrent control of oxidative stress should amplify the health-protective utility of measures which increase endogenous NO production.

In addition to the flavonoids discussed above, practical nutraceutical and lifestyle measures which can boost endogenous NO production include: aerobic exercise training, which via shear stress on vascular endothelial cells enhances both the activation and expression of eNOS;[507-509] increased dietary potassium (see below), which also promotes eNOS activation in endothelium; supplementary citrulline, which works more effectively than supplemental arginine to boost plasma and intracellular arginine levels, thereby offsetting the inhibitory impact of ADMA on NOS activity;[510-514] and dietary nitrate, richly supplied by green leafy vegetables, beets, and beet juice.[515, 516] Recent studies have revealed that dietary nitrate, after absorption, can be secreted in saliva and converted by oral bacteria to nitrite.[517] This nitrite can then be absorbed to boost plasma nitrite levels. Certain iron-containing proteins, such as hemoglobin, myoglobin, and xanthine dehydrogenase, can spontaneously reduce nitrite to NO; propitiously, this reaction is most rapid in tissues that are hypoxic and/or acidic (as thus likely in need of the vasodilatory activity of NO).[516] Intakes of nitrate salts within the feasible dietary range have been shown to exert antihypertensive, platelet-stabilizing, and vasodilatory effects in rodent and clinical studies;[518-522] they also boost the energy-efficiency of exercise (less ATP and O2 required for a given amount of power generation), for reasons that remain unclear, and boost walking distance in intermittent claudication.[523-526] Research on the health impacts of dietary nitrate is just getting under way, so expect a large number of additional protective benefits to be established in coming years. These findings comport nicely with epidemiological studies correlating

increased intakes of green leafy vegetables with decreased risk for heart attack, stroke, and diabetes.[527-534] Spinach, beet juice, and potassium nitrate supplements may be the most practical vehicles for optimizing dietary nitrate intake.

Complementation of Full-Spectrum Antioxidant Therapy with measures that boost endogenous NO production – potentially including aerobic exercise training, quercetin or epicatechin, citrulline, metformin or berberine (discussed in the subsequent section), and increased dietary intake of potassium and nitrate – may prove to have remarkable potential for health promotion.

AMPK Activators – Metformin and Berberine

Another very promising strategy for amplifying the activity of eNOS – and for overall health protection – is activation of the enzyme AMPK-activated kinase (AMPK). This enzyme functions as a detector of "fuel depletion" – increased cellular concentrations of AMP and ADP (which rise when the efficiency of ATP production is compromised) stimulate its activity.[535, 536] An increase intracellular free calcium levels – which can reflect inefficient bioenergetics – also acts indirectly to activate this enzyme. AMPK aids cellular adaptation to suboptimal energy status by boosting the activity of pathways which generate ATP, while suppressing that of anabolic activities which are powered by ATP. One of AMPK's key effects is to activate eNOS by phosphorylating it on two key serine residues; this has the homeostatically appropriate effect of increasing blood flow to tissues that are energetically compromised.[537, 538]

The drug metformin and the herbally-derived nutraceutical berberine, both employed in the treatment of type 2 diabetes, can function clinically as efficient activators of AMPK.[535, 539-542] This appears to reflect the fact that, in clinical tissue concentrations, they modestly impair the efficiency of mitochondrial respiration via partial inhibition of respiratory complex I; the resulting small rise in cellular levels of ADP and AMP leads to AMPK activation. The UK Prospective Diabetes Study, as well as epidemiology, reveal that diabetics treated with metformin are at lower risk for heart attack and stroke than are those treated with other diabetes drugs that achieve comparable levels of glucose control;[543-548] not unlikely, activation of eNOS is a key mediator of this enhanced protection.

However, AMPK activation can work in a number of additional ways to promote longevity and health. AMPK activation is suspected to mediate a portion of the pro-longevity/aging-retardant benefit seen in animals fed calorically-restricted diets throughout life, and AMPK activators may have potential as "calorie restriction mimetics", boosting lifespan and healthspan.[549-553] Diabetics treated with metformin are at lower risk for a number of types of cancer, and this is likely attributable to AMPK activation; AMPK activators are now being evaluated as adjuvants to cancer therapy.[554-563] And there is sound reason to suspect that AMPK activators may reduce risk for diabetes and weight gain; impede the development of ventricular hypertrophy and congestive heart failure; reduce risk or neurodegenerative disorders; and help to preserve the normal structure of bone and cartilage during the aging process.[553] A portion of these benefits may stem from antioxidant effects of AMPK. AMPK helps to keep mitochondria functionally youthful (and hence less prone to generate oxidants) by promoting the complementary processes

of macroautophagy (a "housecleaning" process that breaks down aging mitochondria and other contents of the cellular cytoplasm) and mitochondrial biogenesis (discussed below as an antioxidant strategy).[550, 564-568] AMPK also functions in some cells to discourage the activation of NADPH oxidase, while stimulating at the transcriptional level the synthesis of a number of antioxidant enzymes.[569-575] These effects likely explain why metformin and berberine, which potentially could *boost* oxidative stress via inhibition of mitochondrial respiratory complex I, actually appear to have a net *antioxidant* impact on cells.[576, 577]

Although, to date, metformin and berberine have been employed primarily in the management of diabetes, they may ultimately find their most important use as preventive health aids for the general population. Berberine, long used to treat diabetes in China, may have the most practical potential in this regard, as it is legally available as a nutraceutical rather than a prescription drug. They may be viewed as supporters of eNOS activity, but they also have antioxidant activity, and work in a number of additional ways to promote health and longevity.

Potassium – Antioxidant Electrolyte for Vascular Protection and Bone Health

Diets rich in the electrolyte potassium often have a favorable impact on blood pressure control, and, even when they fail to influence blood pressure, they seem to decrease risk for stroke.[578-584] The vascular protection afforded by potassium-rich diets may be largely attributable to decreased vascular NADPH oxidase activity. Potassium may achieve this in at least a couple of ways.

The electrical charge – membrane potential – across the external membrane of certain types of cells, including vascular endothelium, appears to regulate the activity of NAPDH oxidase. When this charge dissipates – i.e. when the membrane is depolarized – NADPH oxidase activity increases, possibly owing to activation of Rac, a protein which promotes the assembly of the NADPH oxidase complex.[585-588] And membrane depolarization further compromises the protective function of vascular endothelium by reducing its production of nitric oxide; such depolarization suppresses endothelial uptake of calcium, a key activator of the NO synthase enzyme. Conversely, high-normal polarization of endothelial membranes tends to quell oxidative stress while boosting NO production, effects that should be profoundly beneficial to vascular health. Whereas very large increases in blood potassium level tend to collapse membrane potentials, the very modest increases in blood potassium achievable with high-potassium diets have the opposite effect, owing to a stimulatory effect on the membrane "sodium pumps" that kick sodium out of cells. (These "electrogenic" pumps extrude 3 sodium atoms for every 2 potassium atoms they admit, thereby establishing a charge gradient across the membrane.) Hence, such diets tend to decrease endothelial production of superoxide, while increasing that of NO, by modestly elevating endothelial membrane potential.[586, 589-592] (As we have noted, the vascular protection afforded by polyphenols such as quercetin and epicatechin may likewise reflect a small increase in this potential.) Increased production of NO – which is more bioactive owing to decreased superoxide levels – is likely partially responsible for the favorable impact of dietary potassium on blood pressure control and vascular health.[593-596]

In people eating salty diets, high potassium intakes may counteract vascular oxidative stress by an additional mechanism. In salt-sensitive people, salty diets evoke increase production of certain "natriuretic" hormones, such as marinobufagenin (first isolated from the skin of toxic toads!), which boost renal sodium excretion. Although this helps maintain sodium balance and prevent fluid overload, marinobufagenin can act directly on vascular smooth muscle to promote contraction – thereby elevating blood pressure.[597-600] But marinobufagenin also appears to increase production of superoxide by vascular endothelium and certain other tissues, likely via activation of NADPH oxidase.[601-603] By promoting efficient renal excretion of sodium, [604-606] high-potassium diets can lessen marinobufagenin production, thereby alleviating the adverse effects of this hormone on blood pressure and oxidative stress.

Potassium's ability to aid renal sodium excretion arguably should help to counteract a number of other adverse health effects linked to high salt diets. These effects include not only hypertension and stroke, but also congestive heart failure, osteoporosis, kidney stones, and asthma.[607, 608] There is even suggestive evidence that salted diets may play a permissive role in the genesis of dementia.[609] Epidemiologists should devote more attention to the possibly favorable impact of potassium-rich diets on risks for these disorders.

A provocative example of the vascular protection that can be afforded by an ample potassium intake is provided by a controlled clinical study conducted in a Taiwanese home for retired veterans. About half of these veterans were chosen to receive food from a kitchen which used a special potassium-rich salt, in place of ordinary salt, in cooking and at the table; the salt intake of these veterans declined only modestly, but their potassium intake went up by about 78%. In other respects, their diets were comparable to those of the control group. Over the next 31 months of follow-up, the cardiovascular mortality in these veterans was found to be 40% lower than in the controls who continued to receive regular salt.[610]

Diets high in organic forms of potassium – notably, diets rich in fruits and vegetables – have an alkalinizing impact on the body's metabolism that tends to slow the degradation of bone mineral. This explains why post-menopausal women whose diets are rich in fruits and vegetables tend to have denser bones.[611-613] The benefit here is provided, not by potassium per se, but by the negatively charged organic compounds associated with potassium in these foods – the body converts these to bicarbonate, accounting for the alkalinizing effect. Dr. Anthony Sebastian and colleagues at UC San Francisco have shown that regular ingestion of potassium bicarbonate or potassium citrate in a water solution reduces the urinary loss of calcium in women[614, 615] – a benefit that presumably reflects a more alkaline metabolism. This could be particularly beneficial to those who eat a high-protein diet, as sulfur amino acids are metabolized to yield sulfuric acid, which promote bone breakdown.[616, 617] As noted above, such supplementation may also be prudent for those taking supplemental inosine or eating purine-rich diets, to insure that urine remains alkaline so that urate crystals don't precipitate in the kidneys and damage them.

Administering potassium in tablet form is not generally a good idea, as high local concentrations of potassium can be very irritative to the intestinal tract and even cause ulcers. Therefore, potassium is best ingested in potassium-rich foods or drinks. Since potassium bicarbonate in moderate concentrations has a rather mild flavor, it should be feasible to produce potassium bicarbonate-enriched waters and soft drinks to make it more convenient for the general public to boost their intake of protective potassium.[618]

Protective "Carninutrients" with Antioxidant Potential

Certain dietary compounds play physiologically-essential vitamin-like cofactor roles in cellular metabolism, but are not truly nutritionally essential, because they can be synthesized within the body to some extent. Nutrients such are taurine, creatine, and carnitine fall into this category. Remarkably, these three nutrients are provided by animal products, but not plant products; they have thus been dubbed "carninutrients".[619] Vegetarians tend to have lower body stores of these nutrients than omnivores do, and thus they are most likely to benefit from carninutrient supplementation. Although taurine, creatine, and carnitine are not commonly thought of as antioxidants, each appears to exert antioxidant effects under certain circumstances.

Taurine, synthesized from the sulfur amino acids, has an intriguing range of functions in the body. High concentrations are found in muscle and neurons; in cats, which can't synthesize taurine and thus are wholly dependent on a dietary source, severe taurine deficiency leads to blindness and congestive heart failure. In the heart, taurine influences intracellular calcium metabolism in a way that boosts the strength of the heart beat. In fact, high supplemental intakes of taurine have been reported to improve heart function in people who have congestive heart failure,[620, 621] a finding whose credibility is enhanced by the documented utility of taurine in a rabbit model of this syndrome.[622, 623] Taurine also regulates calcium metabolism in platelets, the blood cells that play a key role in clot formation. In some but not all supplementation studies, taurine has exerted a stabilizing effect on platelets complementary to that of aspirin, likely reducing risk for clot formation.[624, 625] The fact that platelets more readily aggregate in vegetarians – one of the few ways in which vegetarians are at greater vascular risk than omnivores – may reflect their poorer taurine status.[619] Supplemental taurine lowers blood pressure in certain rodent models of hypertension, and very limited clinical data are consistent with possible benefit in hypertensive humans, though much more research is required on this point.[578]

Taurine levels are also high in phagocytic immune cells such as neutrophils – and it is here that taurine serves as an antioxidant. Phagocytic immune cells contain an enzyme called myeloperoxidase that uses hydrogen peroxide to generate other oxidants, most notably a highly reactive compound known as hypochlorous acid that helps to kill engulfed bacteria. Unfortunately, hypochlorous acid can also be damaging to the immune cells and bystander cells; nature's solution to this is taurine, which scavenges hypochlorous acid, generating a much less reactive chlorinated taurine molecule in the process – which moreover has anti-inflammatory properties, lessening activation of NF-kappaB.[626, 627] Supplemental taurine has

exerted protective antioxidant effects in many rodent models of inflammatory disorders in which activated immune cells contribute to oxidative stress, presumably by reducing the adverse impacts of excessive hypochlorous acid generation.[628-631] In particular, taurine has been protective in rodent models of atherogenesis, possibly because activated phagocytic cells in the arterial lining (foam cell macrophages) play a pathogenic role in this disorder.[578] Taurine also protects rodents from certain complications of diabetes.[632-636]

Taurine might prove to have particular utility for counteracting the adverse effects of excessive alcohol consumption, as has been demonstrated in rodent models of ethanol feeding.[637-639] While the mechanistic basis of this effect requires clarification, taurine feeding has been shown to increase liver aldehyde dehydrogenase activity in mice, thereby lessening tissue exposure to the toxin acetaldehyde after ethanol ingestion.[640, 641] A small clinical study likewise found that pre-administration of taurine (1.5 g before and again after alcohol) could blunt the rise in blood acetaldehyde following alcohol ingestion.[642] Acetaldehyde is suspected to mediate many of the adverse effects of alcoholism, and may be the chief mediator of such effects in tissues other than the liver.[643-645]

Although taurine is inexpensive and quite safe – it is a key ingredient in "Red Bull" and in many Japanese soft drinks – very few clinical studies have examined its potential for health promotion. This is unfortunate, as it has much more intriguing effects in animal studies than vitamins C or E do. To the extent that supplemental taurine can benefit human health, the effects should be greatest in vegetarians.

Another carninutrient with intriguing properties is **creatine**. High concentrations of creatine are found in skeletal muscle and in neurons, where creatine phosphate serves as a reserve pool of biochemical energy that can be used to rapidly regenerate the "energy catalyst" ATP. Muscles and neurons have rapidly varying energy requirements, which is presumably why they need a creatine phosphate "energy pool" to draw on when energy needs spike up. Creatine also has direct scavenging antioxidant activity for superoxide,[646] and it is conceivable (though not certain) that this contributes meaningfully to antioxidant protection in muscles and neurons. Moreover, under certain circumstances supplemental creatine may have the potential to reduce mitochondrial generation of superoxide by decreasing the electrical potential across the mitochondrial inner membrane.[647] (Creatine's interaction with creatine kinase in the microenvironment of the mitochondrial inner membrane enables rapid re-generation of ADP, whose subsequent conversion to ATP reduces the mitochondrial membrane potential.)

In daily doses of three grams or more, supplemental creatine has been shown to boost creatine stores in skeletal muscle; this improves strength levels in certain types of anaerobic exertion involving rapid repeated contractions.[648] Improved strength may also enable athletes to work harder in training, and thus achieve better increases in muscle mass. For these reasons, supplemental creatine has become quite popular among people engaged in strength training and body building. Creatine loading of muscle has also proved to have a modestly beneficial impact on strength in genetic disorders associated with muscle wasting, such as muscular dystrophy.[649]

Supplemental creatine may also aid blood sugar control in diabetics by aiding the efficiency with which skeletal muscle assimilates glucose.[650, 651] There is some evidence that creatine loading can lower oxidative stress in skeletal muscle, at rest or during exercise; whether this effect is of functional importance is not clear.[652-655] Although creatine plays an important role in the bioenergetics of the heart, creatine supplementation fails to raise heart creatine stores, and no benefit of such supplementation is seen in clinical heart failure.[656, 657]

The impact of supplemental creatine on sports performance has received a fair amount of attention from scientists and the lay public; nonetheless, its greatest benefit to health may stem from its neuroprotective potential. High-dose creatine supplementation has been shown to increase total brain creatine stores by about 9% in humans; the magnitude of this increase is larger in some brain regions than others.[658] In various rodent models of neurodegenerative diseases, supplemental creatine tends to aid neuron survival and mitigate the severity of the syndrome; it is also protective in simulated stroke.[659, 660] It seems likely that improved neuron bioenergetics, as well as an antioxidant effect, contribute to these benefits. The protective impact of dietary creatine in rodent models of Parkinson's and Huntington's diseases has been shown to complement the protection afforded by coenzyme Q10.[661] Of related interest is a recent study in which dietary creatine was found to modestly enhance the average lifespan of aging mice; the authors suspected that creatine's impact on brain function mediated this benefit.[662] Several recent clinical studies find that supplemental creatine can favorably influence cognitive function, most notably in vegetarians; this seems likely to reflect an improvement in neuronal bioenergetics.[663-665]

In brief, there is reason to hope that optimizing brain creatine stores through creatine supplementation will reduce risk for, and perhaps even aid control of, common neurodegenerative disorders such as Alzheimer's or Parkinson's diseases, reduce the brain damage induced by strokes, and modestly benefit cognitive performance. Whether the intriguing protective effects observed in rodent studies will be borne out in clinical trials remains to be seen. Creatine supplementation may be most beneficial for vegetarians, and may be prudent for those seeking optimal neuroprotection.

Yet another intriguing carnitinutrient is **carnitine**. Carnitine plays an obligate catalytic role in the "burning" of fat. Nonetheless, the hope that supplemental carnitine could promote easy fat loss in overweight subjects has not been realized, primarily because in most people carnitine administration can raise muscle carnitine levels only marginally (and then only when if insulin levels are concurrently quite high).[666, 667] However, there is some reason to suspect that supplemental carnitine can accelerate the liver's adaptation to fasting metabolism during the early stages of fasting, aiding hunger control.[668] Accelerated fat burning in the liver may also account for a favorable impact of carnitine on non-alcoholic fatty liver disease.[669] Multigram daily doses of carnitine have been shown to lessen pain in people suffering from cardiac angina or intermittent claudication, owing to a metabolic buffering mechanism that paradoxically promotes selective burning of glucose in poorly oxygenated tissues by boosting pyruvate dehydrogenase activity.[670] The natural derivative acetylcarnitine, in conjunction with lipoic

acid, has been found to "rejuvenate" the function of mitochondria in certain tissues of aging rats, benefiting bioenergetics in these tissues.[671-673] Whether this phenomenon is germane to humans remains to be seen; however, in a recent clinical study, this combination of supplements appeared to reduce elevated blood pressure in people with coronary disease.[674]

There are a number of reports that supplemental carnitine can help cultured cells, rodents, and humans cope with oxidative stress. [675-687] The mechanism of this effect remains obscure. Potential explanations include induction of the key antioxidant enzyme heme oxygenase-1, increased cellular production of mitochondria (as discussed below), and interference with the ability of certain fatty acid metabolites to trigger activation of NADPH oxidase.

More generally, many of the adverse effects of metabolic syndrome and type 2 diabetes are mediated by excess tissue levels of certain fatty acid-derived metabolites – "ectopic fat" – such as diacylglycerol and ceramide, which promote oxidative stress (via NADPH oxidase), inflammation, insulin resistance, and cellular death or dysfunction.[688, 689] In tissues where carnitine availability is normally sub-saturating but can be increased by supplementation, supplemental carnitine has the potential to act as a buffer, diminishing the production of these pathogenic metabolites; this is because fatty acyl-coAs, precursors to these metabolites, can be converted to fatty acyl-carnitines. For example, carnitine administration has been shown to offset the adverse impact of infused fatty acids or a high-fat meal on vascular endothelial function.[690, 691] Perhaps this phenomenon also underlies the utility of acetylcarnitine supplementation in the treatment of diabetic neuropathy,[692] and the reported ability of supplemental carnitine to prevent atherosclerosis in cholesterol-fed rabbits.[693] The impact of supplemental carnitine on risk for the complications of metabolic syndrome and diabetes merit further study.

Body carnitine levels tend to decline beyond age 70, and there are several reports that supplemental carnitine can benefit elderly people complaining of fatigue; moreover, such supplementation also promoted loss of body fat, suggesting that muscle carnitine levels had been functionally sub-saturated.[694, 695] Gerontologists might be well advised to devote more attention to carnitine status and the impact of carnitine supplementation in the elderly.

Glycine – Anti-inflammatory and Antioxidant Amino Acid

Glycine, one of the amino acid building blocks of proteins, has anti-inflammatory and antioxidant activity when consumed in fairly high daily doses (10 grams of more daily). This reflects two key molecular actions. Glycine activates a special receptor in the membranes of many cells - known as a "glycine-gated chloride channel" - that opens a channel through which negatively-charged chlorine atoms can enter cells.[696, 697] This leads to an increase in the electrical potential across the cell membrane, which in turn has implications for cellular function. In the central nervous system, glycine acts as an inhibitory neurotransmitter by activating these channels; this slows the electrical activity of neurons expressing this channel. However, this channel is also found on various types of immune cells, and on the endothelial cells that line the vascular system.[698-700] Ingestion of glycine in sufficient amounts will increase

the activity of the chloride channels in these cells, influencing cellular function. (In contrast, owing to the blood-brain barrier, supplemental glycine has little impact on brain function.)

The types of immune cells influenced by glycine include macrophages and neutrophils. These cells, when activated, produce superoxide via NADPH oxidase; macrophages also produce hormone-like compounds that have immune-stimulant and pro-inflammatory activities. Glycine can act on these cells to reduce their production of both superoxide and pro-inflammatory hormones.[698, 700-702] Hence, glycine has potential utility for controlling acute or chronic inflammatory conditions in which activated macrophages or neutrophils play a prominent role – and this includes most inflammatory conditions.

For example, in rodent studies, oral glycine has been shown useful for preventing inflammatory arthritis, and for quelling inflammatory damage to the liver and lungs.[701, 703-706] Several studies show that glycine can help to prevent or control alcohol-induced liver damage in rats, in which oxidative stress and pro-inflammatory hormones produced by macrophages play a key role.[704, 707-710]

There is an additional way in which glycine can act as an antioxidant – specifically in the liver. The liver is where dietary glycine is broken down and "burned" for fuel; in this process, two molecules of glycine give rise to one molecule of pyruvate. Pyruvate has the remarkable ability to interact spontaneously with hydrogen peroxide, converting it to harmless water.[711, 712] Since hydrogen peroxide is a major mediator of oxidative damage in the liver, it follows that the liver pyruvate derived from a sufficiently high intake of glycine might exert a worthwhile antioxidant effect – independent of modulating chloride channels – just in the liver. In light of the many previous rodent studies demonstrating that glycine has liver-protective potential,[701, 702, 704, 707-710] it may be smart to explore the clinical potential of supplementary glycine in various liver disorders characterized by excessive oxidative stress. This would include such common conditions as hepatitis C, alcoholic hepatitis, and non-alcoholic fatty liver disease.

Another potential use of glycine is in the management of cancer. Tumors need to evoke the formation of new blood vessels – a process known as "angiogenesis" – in order grow beyond a minimal size. Glycine has been shown to slow this process by acting on the endothelial cells that form new vessels; the glycine-gated chloride channels in these cells mediate this effect.[699, 713] In rodents, dietary glycine has been shown to slow the growth of tumors, apparently owing to its inhibitory impact on the angiogenic process.[713, 714] The possibility that glycine might also favorably influence vascular health by exerting a hyperpolarizing effect on vascular endothelium cells has been suggested, and merits evaluation.[715]

Studies in rats suggest that glycine may also have potential in the management of metabolic syndrome. In sucrose-fed rats, 1% glycine in the drinking water reduces blood pressure, triglycerides, and accumulation of abdominal fat.[716] The authors offer evidence that these benefits may reflect an up-regulation of hepatic fatty acid oxidation. Indeed, oral administration of glycine in humans has been reported to provoke a sustained increase in glucagon secretion, an effect which would be favorable to fatty acid oxidation in the liver.[717]

It is convenient to administer high doses of glycine, since this compound is inexpensive, extremely soluble, and has a pleasant sweet taste. A teaspoon of glycine powder weighs about 5 grams, and a reasonable dosing schedule might be a teaspoon 3 times daily, blended into a beverage. Although there seems to have been little interest so far in employing glycine in therapy, a clinical group in Mexico City has reported that oral glycine is useful for preventing "glycation reactions" – a common way in which diabetes damages body organs – in human diabetics as well as diabetic rats.[718-722] This probably reflects the fact that glycine can act as a scavenger for reactive molecules which cause glycation.

Although it would be premature to recommend that healthy people incorporate supplemental glycine into their daily regimens, it may be prudent for people with chronic liver disorders or diabetes to consider this.

Controlling Iron Stores

The "reduced" forms of free iron and copper atoms (the ferrous and cuprous forms that are richest in electrons) can spontaneously donate an electron to hydrogen peroxide or other peroxide compounds to generate the hydroxyl radical, every bit as reactive and dangerous as peroxynitrite. For this reason, almost all of the iron and copper atoms in cells are sequestered in organic complexes that prevent this interaction; nevertheless, a very tiny fraction of these atoms are in a "labile" form capable of interacting with peroxides. The iron content of the liver is so high that iron-catalyzed generation of oxidants plays a pathogenic role in certain liver disorders characterized by increased peroxide production. In patients with chronic hepatitis C, liver iron stores have been found to influence risk for fibrosis and cancer; high iron stores imply greater risk.[723] Conversely, numerous clinical studies by Japanese medical researchers have shown that depletion of liver iron stores with repeated blood drawings (phlebotomy) can reduce liver inflammation and improve response to the major therapy for this disorder, interferon-alpha; one of these studies also concluded that, in the long term, this therapy reduces risk for one of the most lethal complications of hepatitis C – liver cancer.[724-727] Thus, phlebotomy therapy may be appropriate for patients with hepatitis C or other liver disorders associated with chronic oxidative stress. The goal of this strategy is to maintain blood levels of ferritin (an iron-storing protein whose levels are roughly proportional to total body iron stores) in a low-normal range indicative of iron stores that are high enough to avoid anemia or other deficiency symptoms, but low enough to minimize hepatic oxidative stress.

The heme-bound iron found in flesh foods – most notably red meats – is very efficiently absorbed, whereas the non-heme iron supplied by plant products is only absorbed to the extent that the body perceives an increased need for iron. For this reason, vegetarians tend to have relatively low body iron stores, whereas ominivores – particularly those who eat lots of red meat - tend to have high iron stores.[728-730] Whether the lower iron stores of vegetarians provide meaningful protection from oxidative stress, and from diseases associated with oxidative stress – other than in hepatic disorders – is a matter of ongoing controversy. Iron stores also tend to be lower in pre-menopausal women, owing to episodic iron loss via menstruation, and some

scientists suspect that this contributes to greater average longevity in women – though this view is also controversial.

Iron stores tend to be higher in people who have insulin resistance syndrome, and elevated ferritin predicts increased risk for diabetes.[731-736] While these findings could be interpreted as evidence that iron-induced oxidative stress compromises insulin function, and thus helps lead to diabetes, a case can also be made that insulin resistance tends to enhance the efficiency of iron absorption; in other words, high iron levels might be the effect rather than the cause of insulin resistance.[737] While high dietary iron intakes have also been linked to increased diabetes risk, one analysis found that it was only heme-iron intake from red meat – typically high in the saturated fats that promote insulin resistance and diabetes – that was linked with increased risk.[738] The same study failed to observe any reduction of diabetes risk in men who donated blood frequently (and thus presumably would have lower iron stores). On the other hand, one group has reported that phlebotomy therapy improved insulin sensitivity in diabetics with high baseline ferritin levels.[739] Overall, there is not compelling evidence that increased iron stores increase diabetes risk via oxidative stress – but we should keep an open mind on this point, pending future evidence.

Hydroxyl radical generated by ferrous iron in the immediate vicinity of DNA can promote DNA damage that is potentially mutagenic.[366, 740] Thus, there are theoretical grounds for suspecting that increased iron stores may boost cancer risk.[741] Indeed, several reports have shown that serum ferritin levels – roughly proportionate to body iron content – tend to correlate with urinary levels of 8-hydroxydeoxyguanosine, a metabolite produced when DNA is damaged by hydroxyl radicals.[742-744] It has been established that DNA damaged in this way, if not repaired promptly, can give rise to heritable changes in the DNA base code, and hence can contribute to cancer induction.[745, 746]

Not surprisingly, liver cancer risk is greatly elevated in men who have hemochromatosis, a genetic disorder that causes excessive dietary iron absorption. Risk for other types of cancer is also increased, albeit more moderately. Several epidemiological studies over the years have presented evidence that more moderate elevations of body iron stores, in the high-normal range, may also be associated with increased cancer risk – most notably colorectal cancer.[747-751] However, these findings are difficult to interpret, since a diet rich in red meat tends to increase body iron, but can increase cancer risk for other reasons; also, insulin resistance syndrome, which increases risk for many cancers, also may increase the efficiency of dietary iron absorption. Thus, some could argue that correlations between body iron stores and cancer risk simply reflects an association between iron overload and other factors that are the true cause of the increased cancer risk.

A more definitive way to assess the possible impact of body iron stores on cancer risk would be to look at the long-term impact of frequent blood donation. And indeed there have been several reports that cancer rates tend to be lower in blood donors than in non-donors; for example, one such study saw a 21% lower cancer risk in donors.[752] Skeptics note – perhaps justly – that

people who donate blood, and who are accepted for donation of blood, tend to be healthier and more health-oriented than those who don't; so you might expect them to have lower cancer rates. So a more recent study looked at cancer rates within the community of blood donors, seeking to determine whether more frequent donation, or greater total iron removal, correlated with cancer risks. This study found that, whereas frequency of donation per se did not influence cancer risk in this group, men who lost relatively large amounts or iron from repeated donations, as compared to those who lost relatively small amounts, were 30% less likely to develop cancer.[753] These findings suggest that iron loss, rather than donation per se, may be protective. But the most definitive recent evidence in this regard stems from a randomized controlled study which sought to determine whether phlebotomy therapy (blood-drawing every six months), intended to maintain body iron in the low-normal range, would reduce risk for heart attack or stroke in patients with peripheral artery disease. Unfortunately, it didn't – at least, not to a statistically significant extent.[754] However, incidence of new serious cancers ("visceral malignancies") was assessed during a follow-up period of 4.5 years; new cancer incidence was found to be significantly lower – by about one-third - in those receiving the phlebotomy therapy.[755] This appears to be the most definitive evidence available that maintaining body iron in the low-normal range (not associated with any symptoms such as anemia) can reduce cancer risks.

Why does the evidence suggest a role for moderate iron excess in cancer risk, but so far by-and-large fails to incriminate iron in other diseases associated with oxidative stress? Probably because, unless iron levels are grossly high, the oxidants produced by iron interactions constitute a small proportion of the total oxidant load. But, whereas hydrogen peroxide, a mediator of much oxidant-linked disease, is a very weak mutagen, the hydroxyl radical produced by the interaction of free iron and hydrogen peroxide is a very strong mutagen. And just a few key mutations in the DNA of a single cell have the potential to give rise to a life-threatening cancer.

In any case, to the extent that increased body iron stores induce increased oxidative stress that meaningfully increases risk for certain disorders, this risk should be diminished by a vegetarian diet and/or regular blood donations. Studies find that the body iron stories of vegetarians, as assessed by blood ferritin levels, are only one-third to one-half as high as those of omnivores – despite diets that tend to be higher in total iron.

An important caveat, however, is that iron deficiency can increase absorption of the toxic heavy metal cadmium by boosting intestinal expression of proteins which promote iron absorption – but which can likewise expedite cadmium uptake.[382, 386, 756, 757] As noted above, increased body cadmium stores may increase risk for a wide range of health disorders, in part because it promotes oxidative stress. Given the fact that most diets contain toxicologically meaningful amounts of cadmium, maintaining adequate iron status may be importantly protective. Keeping iron stores moderate, but not borderline deficient, may be the most protective policy.

Caloric Restriction and Vegan Diets vs. Mitochondrial Oxidative Stress

As you will recall, mitochondria, the "power plants" of our cells, inevitably produce some superoxide while generating the bioenergy catalyst ATP. Some scientists suspect that mitochondrially-generated oxidant stress plays a role in the aging process, as the rate of mitochondrially superoxide production is far greater in short-lived species than in longer-lived ones.[758] Intriguingly, caloric restriction – feeding animals only 60-70% of the daily calories that they would ingest if given free access to food – not only slows the aging process and increases maximal lifespan, but it also slows the rate of mitochondrial superoxide production.[759-761] A similar effect has been reported in animals fasted on alternate days, or fed a diet low in methionine – strategies which likewise increase maximal lifespan in rodents.[762-764] Vegan diets of modest protein content tend to be relatively low in methionine,[765] and modified alternate day fasting, "carb-concentrated diets"[766], and meal skipping may have practical potential as strategies for achieving moderate sustained reductions in calorie intake.[767-770] It would be of interest to determine whether such reasonably feasible dietary regimens could impact mitochondrial oxidant production in humans. Whether or not they accomplish this, they should promote leanness and good health.

Vegan diets of moderate protein content are associated with a down-regulation of hepatic IGF-I synthesis, likely reflecting a modest degree of essential amino acid restriction.[771-776] Such diets, in the long term, also tend to down-regulate insulin secretion, owing to favorable effects on fat mass and insulin sensitivity; the resulting reduction in circulating insulin tends to further decrease systemic IGF-I bioactivity by boosting hepatic production of IGFBP-1.[777-779] This phenomenon may be largely responsible for low risks for "Western" cancers observed in Third World societies whose traditional diets are quasi-vegan.[780, 781] However, decreased systemic IGF-I activity could also be expected to increase the transcriptional activity of FOXO transcription factors in many tissues.[782, 783] Recent research reveals that, in human endothelial cells, FOXO3a boosts the transcription of a range of genes that protect mitochondria from oxidative stress, including the manganese-dependent (mitochondrial) superoxide dismutase, catalase, and UCP-2.[784-788] (Conceivably, this phenomenon might be largely responsible for the reduction in mitochondrial oxidant production noted in calorically-restricted rodents, in which IGF-I bioactivity is notably decreased.) As noted, vegan diets tend to be modestly methionine restricted, and methionine restriction in rodents somehow suppresses superoxide production by complex I of the mitochondrial ETC.[789] Hence, there is some reason to suspect that vegan diets of moderate protein content, independent of their phytochemical content, will exert an antioxidant effect associated with decreased mitochondrial oxidant production and increased mitochondrial antioxidant protection.

It should be noted that each of the components of Full-Spectrum Antioxidant Therapy can be expected to protect mitochondria from oxidant stress: PhyCB, because it will antagonize the "kindling" mechanism whereby oxidants produced by NADPH oxidase can damage the mitochondrial ETC and amplify its capacity for superoxide generation; AX, because it is the premiere natural antioxidant for cellular membranes, including those of mitochondria; high-

dose folate and CoQ, because they are effective and versatile mitochondrial oxidant scavengers; and lipoic acid and NAC, because they boost production of the mitochondrial antioxidant glutathione. Moreover, lipoic acid will help protect mitochondria from external oxidants by its inductive effect on various antioxidant enzymes.

Promoting Mitochondrial Biogenesis as an Antioxidant Strategy

When the number of mitochondria – or rather of mitochondrial electron transport chains – is increased per unit of tissue volume, the same rate of ATP production can be maintained while mitochondrial superoxide production is reduced. This is because the ETCs will be less glutted with electrons, as the electron flow that generates ATP will be spread over a larger number of ETCs. Electrons are most likely to "leak out" to form superoxide when electrons get backed up in the ETC – much like cars backed up in a horrible traffic jam are more likely to take off-ramps!

Remarkably, Dr. Bruce Ames and colleagues have discovered a practical nutraceutical strategy for increasing cellular production ("biogenesis") of mitochondria. Supplementing with a combination of lipoic acid and acetylcarnitine (a key natural metabolite of carnitine) somehow accomplishes this trick in the tissues of aging rodents.[790-794] It is not yet clear how this works, or why it is only effective in older animals; however, increased production and activation of a cell regulatory factor that promotes mitochondrial biogenesis, PGC-1alpha, likely plays a key role in this effect.[795-798] One of the most recent rodent studies of this regimen showed a favorable effect on cognitive function in mice prone to an Alzheimer's-like syndrome.[799] If this strategy proves applicable to humans, it holds out the prospect that, as we age, our tissues will be able to produce ATP more efficiently, while oxidative stress is kept under better control. Although many people are now using this supplementation regimen, clinical studies to confirm its efficacy and define the most effective dose levels are still needed. However, in a controlled pilot clinical trial enrolling patients with coronary heart disease, joint administration of R-lipoic acid and acetylcarnitine tended to lower systolic blood pressure and increased the diameter of a major arm artery (brachial artery); whether these benefits reflected increased mitochondrial biogenesis is not clear.[800] Hopefully further clinical studies evaluating this strategy will soon be forthcoming. In particular, it would be intriguing to know whether this approach might improve exercise capacity in the elderly.

As we have noted previously, dietary quercetin can markedly boost mitochondrial biogenesis in the skeletal muscles of mice[489] – an effect probably mediated by increased endothelial production of nitric oxide. Some studies suggest that it may also have this potential in humans – albeit to only a very modest degree.[490] And, as you will recall, agents which activate AMPK, such as metformin and berberine, likewise have the potential to boost mitochondrial biogenesis.[550, 568]

Uncouplers as Mitochondrial Antioxidants

With respect to controlling mitochondrial stress, it is intriguing to cite a simple and inexpensive pharmaceutical strategy that may have potential in this regard – albeit it is unlikely to ever achieve legal approval. The drug dinitrophenol (DNP) is known as an "uncoupling agent" because it enables high energy electrons to flow down the mitochondrial electron transport chain without the need to couple this to increased production of the bioenergy catalyst ATP; the energy of these electrons is released as heat. While uncouplers can have an adverse effect on tissues that have a high ATP requirement, they also markedly quell mitochondrial generation of superoxide. Indeed, our cells are capable of making proteins that can function as antioxidants by producing mild mitochondrial uncoupling.[801, 802]

In the 1930s, Harvard physicians discovered that, in a daily dose of 3-5 mg/kg, DNP greatly boosted metabolic rate and enabled obese patient to lose weight at a dramatic pace, usually without discernible side effects.[803] DNP quickly became a popular sensation – the first really big weight loss drug, and definitely the most effective – but its promise was tarnished when some people who exceeded the recommended dose developed persistent hyperthermia that in some instances proved fatal.[804] Since the margin between the effective dose and the potentially lethal dose was rather narrow, the recently established FDA banned it. (However, some serious bodybuilders continue to use it to this day, albeit "under the table" – DNP as a raw chemical is readily available and inexpensive; case reports of fatal DNP-induced hyperthermia are still appearing.[805-807])

In recent years, mitochondrial uncouplers have attracted the interest of longevity scientists because of their ability to decrease mitochondrial supeoxide generation. Indeed, very low dose of DNP were shown to increase average lifespan in yeast and fruit flies.[808, 809] These findings motivated a recent remarkable study in which, beginning at 18 weeks of age, mice were administered the strikingly low dose of 1 mg DNP per liter in their drinking water.[810] This dose did not cause weight loss, but it significantly blunted weight gain, and these leaner mice had better insulin sensitivity than controls. Much more striking were the remarkable reductions in markers for oxidative DNA and protein damage in the tissues of these mice – most dramatic in the brain, where oxygen consumption was notably enhanced – observed after 5 months of DNP feeding. And average longevity of these mice was about 7% greater than that of controls – a modest but statistically significant increase. If these surprising findings prove to be replicable, could "mini-dose uncoupler therapy" have clinical potential as an antioxidant, cancer-preventive, and healthspan extending strategy?

Risk Factor Control – Statins and Angiotensin Antagonists

The adverse effects of many well-accepted cardiovascular risk factors – such as elevated LDL cholesterol, hypertension, hyperglycemia, the free fatty acid excess associated with metabolic syndrome, hyperhomocysteinemia, and high-salt diet – are mediated in large part by induction of oxidative stress in vascular endothelium and other cells.[811-829] It therefore follows that pharmaceutical or nutraceutical measures which moderate these risk factors have an antioxidant

impact. Statins and angiotensin antagonists are particularly notable in this regard, in that, independent of their impacts on LDL cholesterol or blood pressure (respectively), they have the potential to act directly on vascular endothelium and other tissues to lessen activation of NADPH oxidase;[830-835] this may help to account for their versatile clinical utility. These considerations serve to underline the likelihood that effective nutraceutical antioxidant strategies, such as those recommended here, may potentiate the beneficial impact of therapies that modulate vascular risk factors, and may be particularly helpful for patients in whom risk factors cannot be fully optimized.

Oxidative Stress and Longevity

What impact could we expect Full-Spectrum Antioxidant Therapy to have on the aging process? As you may know, the "free radical theory of aging" maintains that the rate at which our cells generate oxidants plays a crucial pace-setting role in the aging process, in part because these oxidants induce cumulative mutagenic damage in our cellular DNA (nuclear and mitochondrial), as well as structural damage in long-lived proteins such as collagen.[836] And it certainly is not sheerly accidental that the rate of mitochondrial oxidant generation in a species tends to be inversely proportional to its typical lifespan; thus, the mitochondria of rat cells generate superoxide several times more rapidly than those of human cells.[837, 838]

The free radical theory of aging is rooted in the notion that accumulation of random errors in DNA or key proteins drives the aging process. This view has some intuitive appeal, but is unlikely to represent the whole truth. It may be more appropriate to view aging as just another phase of the developmental process that begins with fertilization of the ovum. Molecular biologists still have only a hazy understanding of the incredibly intricate pre-programmed interactions that enable a fertilized egg to develop into an embryo, or an infant into a young adult. But it is clear that, with perhaps a few exceptions (such as the "intentional" DNA scrambling that gives us immune cells that can attack a wide array of targets) random error has little to do with this process! And so it seems likely that the aging process – characterized by a slow but steady decline in the maximal physiological capacities of our body organs, a loss of tissue elasticity, and cosmetic changes such as graying and wrinkling – is also a pre-programmed part of our development, that would occur even in the absence of significant oxidative stress or age-related disease. Think of it as Nature's version of the "programmed obsolescence" which Detroit automakers were accused of building into their cars! And, unless we decide to forego procreation, aging and death is necessary to insure that there will be room for the emerging younger generation; what is tragic for the individual may be essential for the species.

Viewed from this perspective, it doesn't seem likely that effective antioxidant measures will deter the aging process or markedly change maximal longevity. The strategies which slow aging and increase maximal lifespan in rodents do indeed lessen oxidative stress – but they also decrease growth factor activities (such as insulin-like growth factor-I) which seem to play a pace-setting role in the aging process.[839, 840] At least so far, antioxidant chemicals haven't

succeeded in increasing maximal lifespan in rodents. A provocative study found that mice bioengineered to express increased levels of the antioxidant enzyme catalase in their mitochondria achieved a 5-month increase in average and maximum lifespan[841] - but the impact of caloric restriction on rodent lifespan can be considerably greater. A decrease in oxidative stress may indeed be *necessary* for a longer lifespan, but it is unlikely to be *sufficient*. And the reason why short-lived species like mice have comparatively high background oxidative stress is because their level of oxidative stress doesn't notably impact their ability to produce viable progeny, since they live for at most a few years; thus, there is no selection pressure to suppress this oxidative stress. In contrast, if the cells of a human infant generated oxidants at same rate as those of a mouse, he would be unlikely to reach reproductive age before dying of oxidant-induced cancer or oxidant-mediated organ failures. So humans have evolved the superior oxidant control that gives most of them a fighting chance to procreate and survive for the Biblical three-score and ten years.

The good news is that, even if antioxidant measures don't slow the fundamental aging process or increase our *maximal* lifespan, they may well help us to *age gracefully* and achieve a higher *average* lifespan by helping to ward off or delay a wide range of age-related diseases and deficits of organ function that are induced or exacerbated by oxidant stress, and that aren't truly intrinsic to aging. In other words, effective antioxidant strategies, in conjunction with other prudent health-promoting behaviors, stand a good change of markedly increasing our *healthspan* – the number of years we live in reasonably good health with adequate physical capacities. Being ninety years old won't be all that bad if we don't have cancer, heart disease, or diabetes, if our mental acuity, sight, and hearing – if not as sharp as they were at age at age 20 – are still reasonably intact, and if we can still exercise regularly, make some useful contribution to our society, and enjoy many of the good things that make life worth living.

It is heartening to realize that there have been some human societies in which heart attack, diabetes, even hypertension, stroke, and dementia were (or are) extremely rare in the elderly, and in which many cancers common in "Western" society are far less common.[842-847] A gradual decline in our maximal physical capacities may be inevitable, but many of the disorders and infirmities which plague old age are not. Ideally, after living a very long, productive, and reasonably healthy life, we can expect to succumb to an injury or infection that, in our younger years, our more youthful physiologies might have coped with. That is the best that we can reasonably hope for – and we can markedly increase our chances of achieving such a full, blessed life by eating, exercising, and supplementing in a smart, self-protective way, and by having vocations, relationships, and interests that give us a good reason to get up in the morning.

Resources

The nutraceuticals discussed in this monograph are of course available from many distributors worldwide. However, ingesting high doses of spirulina or of folic acid can present a logistical challenge. My small company nutraceutical company NutriGuard Research has therefore

developed a couple of products (*Chocolatl Verde* and *AquaFol*) which makes this more feasible, and which can be shipped worldwide via the U.S. Postal Service. They can be contacted via phone (800-433-2402/760-942-3223) or the net: http://www.nutriguard.com/vitamin-store/.

Appendix – A Few More Protective Nutraceuticals

An effective strategy for coping with oxidative stress may not be the definitive "cure" for any particular disorder, but it could be expected to delay the onset and mitigate the severity of a very large number of maladies. Nonetheless, it is evident that antioxidants are not the only food factors that can make a worthwhile contribution to health preservation. Here's a brief overview of a few additional nutrients and phytochemicals which have gone unmentioned in the above discussion, but which have broad health-promoting potential as supplementary nutraceuticals:

Vitamin D – Vascular Protection, Bone Health, and Cancer Prevention

"Nutritional insurance formulas" are supplements designed to insure adequate or optimal intakes of the nutritionally essential vitamins and minerals.[848] Among the nutrients provided by well-designed nutritional insurance formulas, **vitamin D** now appears to have exceptional promise for health promotion. It has long been recognized that vitamin D is required for maintenance of bone density, but more recent research has correlated good vitamin D status with reduced risk for a number of types of cancer, vascular disorders, and autoimmune conditions such as multiple sclerosis and type 1 diabetes.[849-851] Vitamin D is actually more properly considered a hormone, or hormone precursor, than a nutrient. Unless you eat liver or fish liver oils, the vitamin D content of a natural, unsupplemented diet is negligible. Most of the vitamin D in our body is produced endogenously in our skin, in a reaction between UV light and an intermediate in cholesterol synthesis, 7-dehydrocholesterol. 30 minutes of whole-body sun exposure during the summer – when sunlight is rich in UV – can produce as much as 20,000 IU of vitamin D – also known as cholecalciferol. This is rapidly converted by the liver to 25-hydroxyvitamin D; blood levels of this compound can be used to assess vitamin D status. 25-hydroxyvitamin D has little direct hormonal activity, but a certain proportion of it is converted by the kidneys and certain other tissues to the hormonally active form of vitamin D, known as calcitriol. The key essential function of calcitriol is to promote efficient dietary absorption of calcium. Since blood levels of calcium must be maintained within a very narrow range, the production of calcitriol by the kidneys is very carefully regulated in line with need; in other words, low blood calcium triggers a boost in calcitriol production, whereas high blood calcium has the opposite effect.

Although most of the calcitriol in the body and blood derives from regulated production within the kidneys, many epithelial tissues capable of giving rise to cancer can make their own small amounts of calcitriol, and the rate at which they make it is directly proportional to circulating levels of 25-hydroxyvitamin D.[852, 853] Therefore, calcitriol activity in these tissues is determined largely by 25-hydroxyvitamin D levels, which in turn reflect recent exposure to UV-rich sunlight. In many of these epithelial tissues, calcitriol activity has an anticarcinogenic effect, for reasons that are a bit too complicated to discuss here. These new understandings have encouraged medical epidemiologists to investigate the impact of UV exposure on risks for many cancers. Not surprisingly, they have concluded that many cancers are more common among people who live at high latitudes – where the UV content of sunlight is negligible during winter

months – or in areas where air pollution limits UV exposure. Dr. Bill Grant, an environmental expert who has applied his skills to medical epidemiology, has estimated that over 23,000 people in the U.S. annually die prematurely from cancer owing to suboptimal vitamin D status reflecting inadequate UV exposure.[854] More recently, his work points to a major influence of vitamin D status on survival in people with pre-existing cancer;[855] this likely reflects the fact that calcitriol can act on many cancers to slow their growth. The protective impact of good vitamin D status on risk for colorectal cancer – second only to lung cancer as a cause of mortality - has been especially well documented.[856, 857] But risks for at least 16 other types of cancer have correlated inversely with estimated UV exposure or vitamin D status in in published studies.[858]

Remarkably, the link between increased sunlight exposure and decreased risk for internal cancers was first made by Peller and Stephenson in 1937, who reported that, whereas sailors in the U.S. Navy had eight-fold higher rates of skin cancer than the general population, they were only 40% as likely to develop internal cancers.[859] This work was followed up by Apperly in 1941, who was the first to report that rates for many cancers rose with increasing distance from the equator; he rightly concluded that "solar radiation" was exerting a protective effect. But the Garland brothers of San Diego, Cedric and Frank, may have been the first to identify vitamin D as the mediator of this sunlight-mediated protection, when they pointed to a possible role of vitamin D in colon cancer prevention in 1980.[860]

The lower risk for certain autoimmune disorders associated with increased UV exposure may reflect the fact that activation of certain immune cells that are master regulators of the immune response (antigen-presenting cells) boosts their capacity to generate calcitriol; this calcitriol provides a feedback signals that decreases their activation in some respects.[861-863] This feedback mechanism evidently will work more avidly when circulating levels of 25-hydroxyvitamin D are higher. This anti-inflammatory effect of vitamin D may also contribute to the favorable impact of good vitamin D status on vascular risk. Calcitriol activity in antigen-presenting cells also has the effect of increasing their capacity to kill engulfed bacteria, while boosting their production of a natural antibacterial protein known as cathelicidin.[864, 865]

As if these benefits were not enough, other evidence suggests that effective vitamin D activity may help to prevent heart attack, stroke, diabetes, hypertension, and congestive heart failure.[866-875] These effects may be at least partially attributable to vitamin D's ability to suppress production of parathyroid hormone – an effect which is also the key to its favorable impact on bone density. Although the chief targets of parathyroid hormone's physiological activity are bone and kidney, in moderate excess it can have an adverse impact on the function of a number of other tissues; these effects are seen more dramatically in people with primary hyperparathyroidism, in whom benign tumors generate a continual excess of parathyroid hormone.

Although vitamin D is manufactured in our skin, orally administered vitamin D is absorbable, and can provide comparable benefit. This is very important – particularly because, in the winter

months, the UV content of light is so low in northern latitudes that even whole-body sun exposure will produce very little if any vitamin D. Indeed, you could lie naked on Boston Commons on a clear day in mid-winter for 8 hours and make hardly any vitamin D at all! Under these circumstances, people have to rely on vitamin D stored in their fat cells, until UV exposure becomes meaningful again during the spring. Evidently, it would be sensible to buffer low winter levels of vitamin D by taking this vitamin in supplements. And supplemental vitamin D would be of particular value to shut-ins who get minimal sun exposure, to people with heavily pigmented skin (who don't manufacture vitamin D as efficiently as those with light skin), or to women who won't expose their skin to the sun for religious reasons (vitamin D deficiency is rife in many sunny Muslim lands!) Elderly people are also good candidates for vitamin D supplementation, since their thinner skin has a lower capacity to manufacture this vitamin.

Nonetheless, the potential of supplemental vitamin D to promote health has barely been tapped, owing to a catastrophic screw-up by nutritional scientists. In an excess of caution, they long ago set the RDA for vitamin D at 400 IU for adults – a dose just barely high enough to prevent rickets, a bone disorder seen in severe vitamin D deficiency. But you will recall that humans can make up to 20,000 IU of vitamin D daily via sunlight exposure. So the tiny doses of vitamin D provided in most multivitamin pills would fall far short of optimizing vitamin D's potential for health protection.[876-878] In retrospect, the decision to set such a low RDA for vitamin D may have led to hundreds of thousands of premature deaths from cancers and vascular disorders among people who chose to use vitamin supplements, but failed to get a meaningful boost in their vitamin D status.

Even for people who live in sunny climes, getting one's primary vitamin D nutrition from supplements may be preferable to intentional UV exposure. Even though regular (as opposed to episodic) UV exposure does not appear to increase risk for the most serious, life-threatening type of skin cancer, melanoma, it does increase risk for nuisance skin cancers and also promotes cosmetic aging of skin. Taking effective supplemental doses of vitamin D should enable you to maintain good vitamin D status, while avoiding the cumulative UV-mediated skin damage that ages your appearance.

In light of current evidence, a daily supplemental intake of 2,00-5,000 IU vitamin D appears prudent. This is a safe level of intake that can be expected to promote excellent vitamin D status. Supplemental vitamin D is believed to be safe for adults in daily intakes up to 10,000 IU – and even this may be a conservative safety limit. Dr. Reinhold Vieth, one the world's chief experts on vitamin D metabolism, states that the lowest daily dose of vitamin D reliably reported to induce vitamin D toxicity in an adult was 40,000 IU.[877]

Vitamin K for Healthy Bones, Arteries, and Livers

Another fat-soluble vitamin which may have important implications for health – but which so far has received relatively little popular attention – is vitamin K. The chief food form of this vitamin is K1 – phylloquinone; dark green leafy vegetables are typically good sources of this

compound. The structurally similar vitamin K2 (a.k.a menaquinone), produced by our gastrointestinal bacteria, can also be absorbed, and is found in certain fermented food products such as natto (fermented soy beans). Although vitamin K has long been known to be essential for proper clotting mechanisms (anti-coagulant drugs such as Coumadin antagonize its activity in this regard), vitamin K is also needed for the proper production of the protein osteocalcin that plays a key role in bone metabolism. Vitamin K enables a structural modification of proteins known as "carboxylation". An increase in the blood level of uncarboxylated osteocalcin, or in the ratio of uncarboxylated osteocalcin to the properly carboxylated form, is indicative of vitamin K deficiency, and correlates with lower bone density and increased fracture risk.[879, 880] Since at least 90% of circulating osteocalcin is carboxylated even in people with average vitamin K nutrition, it seems likely that uncarboxylated osteocalcin is acting as a functional antagonist of the properly carboxylated form – in which case, minimizing uncarboxylated osteocalcin levels can be expected to pay off in improved bone health. (Alternatively, vitamin K might have some important, still undiscovered function in bone, and undercarboxylated osteocalcin is serving as a sensitive marker or vitamin K status.) Studies show that supplemental vitamin K decreases uncarboxylated osteocalcin levels dose-dependently up to 1,000 mcg vitamin K daily[881] – whereas the current recommended daily dietary intake of vitamin K (suitable for maintaining adequate levels of clotting proteins) is 90 mcg in women and 120 mcg in men. These considerations suggest that ordinary diets are unlikely to provide sufficient vitamin K activity for optimal bone health.

A number of studies have correlated poor vitamin K status with lower bone density and increased fracture risk.[882, 883] Moreover, several long-term clinical trials (1-3 years in duration), most of them Japanese studies employing vitamin K2, have found that supplemental vitamin K can aid maintenance of bone density and decrease fracture risk.[884] The impact on fracture risk in these studies, which were of 1-3 years duration and employed a very high dose of vitamin K2 (menaquinone-4), 45 mg, is astounding – on average, a 40% reduction in spinal fracture risk, and an 80% reduction in risk for other types of fractures – including those of the hip, so dangerous for elderly women. Although vitamin K supplementation in these studies also modestly boosted bone density, the decrease fracture risk is disproportionately high, and suggests that improved vitamin K status can have prompt and major impacts on bone structural integrity that are independent of bone mineral content per se. Other studies conclude that the efficacy of vitamin K for promoting bone health appears to be complementary to that of drugs commonly used for this purpose, such as bisphosphonates and raloxifene.[885]

The Japanese interest in menaquinone stems from evidence that risk of hip fracture in provinces of this country correlates directly with the extent to which natto is consumed in these provinces; there is an east—west gradient in natto consumption that parallels a gradient in hip fracture risk![886, 887] A 100 gram serving of natto provides about 1.3 mg of vitamin K2, so it is reasonable to assume that daily vitamin K intakes of this magnitude will have a very worthwhile impact on fracture risk. Unless you are overtly vitamin K deficient, or taking drugs that antagonize vitamin K activity, getting extra vitamin K from supplements or a "greener" diet won't increase your production of clotting factors or risk for a heart attack or stroke, and so is likely to be quite

safe. However, if your doctor has prescribed Coumadin for decreasing your clotting activity, it is important to avoid marked variations in your daily vitamin K intake, so that your doctor can prescribe a dose of Coumadin that will be continuously appropriate.

Recent studies indicate that good vitamin K status may do more than enable proper clotting and promote bone health.[888] Vitamin K promotes carboxylation of a protein in the vascular wall (MGP) that helps prevent inappropriate calcification of arteries.[889] Epidemiological studies suggest that vitamin K2 may be more effective than vitamin K1 in this regard.[890] People with relatively good vitamin K2 nutrition appear to be at lower risk not only for vascular calcification, but also for death from coronary heart disease.[891, 892] In addition, a 3-year controlled clinical study found that vitamin K2 supplementation helped preserve the youthful elasticity of the carotid artery – consistent with the expected favorable impact on vascular calcification.[893] Hopefully future controlled studies will evaluate the impact of supplemental vitamin K on risk for heart attack and stroke.

Vitamin K may also have potential for prevention and control of liver cancer. Many liver cancers make an undercarboxylated form of the clotting factor prothrombin; this reflects the fact that these cancers have a diminished capacity to use vitamin K for carboxylation reactions. Remarkably, this undercarboxylated prothrombin can act as a potent growth factor for many of the liver cancers that produce it; normally carboxylated prothrombin lacks this growth factor activity.[894, 895] Fortunately, increased intakes of vitamin K promote increased carboxylation of prothrombin in these cancers, so that production of the undercarboxylated growth factor is suppressed.[896, 897] These considerations suggest that ample intakes of vitamin K might help to prevent or slow the growth of many liver cancers – and that is precisely what medical research is confirming. Vitamin K administration often slows the growth of hepatic tumors in mice.[897, 898] A controlled study found that, in women who had chronic hepatitis C and were thus at high risk for liver cancer, those supplemented with vitamin K2 had a much lower subsequent incidence of liver cancer than those who did not.[899] Other studies have examined the impact of vitamin K2 supplementation in patients whose liver cancer had gone into remission after successful initial treatment; most, though not all, of these studies have concluded that vitamin K can slow cancer recurrence in these patients and increase their average survival.[900-902] Remission of liver cancer following vitamin K supplementation has also been described occasionally, though in most cases vitamin K is no magic bullet for this deadly disease.[903, 904] These findings, while not yet conclusive, suggest that people with chronic inflammatory liver disorders (such as viral or alcoholic hepatitis), as well as those who have already developed liver cancer, would be well advised to maintain optimal vitamin K status. Owing to the fact that many liver cancers have a diminished capacity to use vitamin K , relatively high doses of this vitamin might be required for optimal protection in this regard.

Protective Minerals – Calcium, Magnesium, and Strontium

Supplemental **calcium**, while no panacea for prevention of osteoporosis, has a well-defined role for delaying bone loss; like vitamin D, it suppresses the production of parathyroid hormone, a

hormone which helps to maintain adequate blood calcium levels by promoting breakdown of bone mineral.[905] A recent meta-analysis concludes that supplementing with calcium plus vitamin D does indeed tend to lower fracture risk in postmenopausal women – whereas vitamin D alone is ineffective in this regard.[906] (Arguably, the modest doses of vitamin D employed in these studies might have impacted that latter conclusion.) The impact of dietary calcium may actually be greater in childhood, when dietary calcium can notably influence the peak bone mass that is achieved.[907] A further benefit of supplemental calcium is that it may decrease risk for colorectal adenomas, some of which progress to colorectal cancer.[908]

However, recent analyses of controlled clinical trials of calcium supplementation have observed a modest (about 18%) but statistically significant increase in heart attack risk in those receiving calcium.[909, 910] Although this conclusion has been challenged,[911] the results are not entirely implausible for at least a couple of reasons. One is that supplemental calcium can impede absorption of concurrently ingested magnesium; as noted below, good magnesium status is vital for vascular health.[912] Within "health food" circles, it is commonly recommended that supplemental calcium should be "balanced" with a supplemental intake of magnesium about half as high – whereas most pharmaceutical calcium supplements promoted for bone health contain no magnesium. (In contrast, high calcium foods, which have not been linked to increased heart attack risk, typically also supply magnesium.) But another potential drawback of a high calcium intake is that it can lower blood levels of the activated form of vitamin D, calcitriol. This in turn has the potential to boost renal production of the hormone renin, which plays a role in the genesis of cardiovascular disease and hypertension.[913, 914] Whether this is an important effect is not yet clear; in any case, the impact of supplemental calcium on vascular health requires further evaluation.

Magnesium, which can function intracellularly as a mild calcium antagonist, is crucial for proper cardiovascular function, and probably has received far less clinical research attention than it merits. Poor magnesium status appears to increase risk for dangerous disorders of heart rhythm (arrhythmias), renders platelets more likely to form clots, and can also be a contributory cause of high blood pressure;[915-917] recent clinical studies by Dr. Michael Shechter are at long last establishing a role for magnesium supplementation in the treatment of patients with coronary disease.[918-921]

Assuring optimal intakes of certain trace minerals via supplementation may also confer benefits. **Manganese** plays an essential role in the production of mucopolysaccharides, which are critical components of connective tissues such as bones and cartilage; if you are a sports fan, you may remember that the physicians of star NBA center Bill Walton concluded that manganese deficiency was a factor in the failure of his foot to heal properly. In a two year double-blind clinical study, in which postmenopausal women received calcium, a trace mineral supplement (with zinc, manganese and copper), a combination of the two, or placebo, only those receiving the calcium *and* trace minerals achieved an increase in spinal bone density.[907] Bioavailable forms of **chromium** such as chromium picolinate appear to have a favorable impact on impaired insulin sensitivity in at least some people[922-925] – Chinese diabetics appear to get

particular benefit in this regard[926] – and a recent clinical study has found that this mineral promotes appetite control in women who have carbohydrate cravings.[927] However, promotional claims that supplemental chromium can exert a favorable effect on body composition have not been borne out by subsequent research. Other nutritional minerals with potentially beneficial health impacts – but which so far have received little study – include **silicon**[928-932] and **boron**.[933, 934] The essential antioxidant role of selenium has been discussed above.

Recently, the mineral **strontium** has emerged as a potent aid to bone health. Although strontium does not appear to be nutritionally essential, it occurs naturally in water and in foods, is reasonably absorbable, and tends to accumulate in bone mineral. This effect was a matter for concern when radioactive isotopes of strontium were produced by nuclear testing during the Cold War. However, the stable form of strontium that predominates in our environment appears to be quite safe, and recent animal and clinical studies reveal that absorbable strontium complexes can promote increases in bone density; this reflects an inhibitory effect of strontium on the bone-resorbing activity of cells known as osteoclasts, coupled with an increase in the production of osteoblast cells that generate bone matrix.[935, 936] A patented form of strontium, known as strontium ranelate, has been developed as a drug for treating postmenopausal women experiencing osteoporosis; two large multinational studies have shown that, in daily doses providing 680 mg of strontium (2 g of strontium ranelate), prolonged administration of this agent reduces risk for vertebral fractures by over one-third, and risk for the most common non-vertebral fractures by nearly 20%.[937-939] These benefits are associated with progressive increases in bone density of 3-5% per year. Lower doses of this agent also improve bone density, though to a lesser degree.[940] In doses up to 2 g daily, strontium ranelate appears to be quite safe and well tolerated.

Since the benefit of strontium ranelate appears to stem from the strontium per se, it seems highly likely that other absorbable complexes of strontium, such as the lactate, citrate, or carbonate salts, will also be protective; in the U.S., these are legally available as nutraceutical supplements. A total daily intake of 680 mg strontium is typically recommended. Although controlled clinical studies of strontium supplementation to date have focused on postmenopausal women at high risk for fracture, there is no reason to suspect that strontium would not also benefit elderly men who are developing osteoporosis, and it also seems likely that, perhaps in more modest doses, strontium supplementation in younger men and women might have potential for prevention of osteoporosis. There is limited evidence that exposure to strontium in drinking water may aid prevention of dental caries in young people;[941, 942] moreover, cell culture studies as well as clinical observations hint that strontium might also have some potential for preventing the loss of cartilage associated with osteoarthritis.[943-945] In any case, strontium supplementation appears to have a bright future in preventive health.

Omega-3s for Your Brain and Heart

With respect to essential fatty acids, diets which contain at least modest amounts of natural plant oils are likely to insure adequate intakes of the "omega-6" fatty acids. On the other hand,

many diets contain suboptimal amounts of the omega-3 fats – the **alpha-linolenic acid** found in some oils and nuts (flax oil and walnuts, for example), and the longer chain omega-3s, **EPA** and **DHA**, provided by marine fish and fish oils. DHA plays a key structural role in the brain – and diets poor in omega-3 nutrition have been linked to increased risk for depression and suicide.[946-948] The retina can be considered an extension of the brain, and DHA is important for the proper function of photoreceptor neurons and retinal health.[949-952] In fact, good omega-3 status tends to correlate with reduced risk for macular degeneration, the primary cause of partial blindness in the elderly;[953, 954] a major clinical study, AREDS2, is now assessing whether supplemental EPA/DHA can slow the onset and progression of this disorder.

Good omega-3 nutrition appears to reduce risk for so-called "sudden death" cardiac arrhythmias, and has also been linked to lower levels of markers of systemic inflammation that correlate with increased vascular risk, such as C-reactive protein.[955-959] In the Italian GISSI study, in which patients who had previously suffered a heart attack were randomized to receive 1 g daily of EPA/DHA or placebo, risk of cardiovascular death was 17% lower during two years of follow-up in those receiving the fish oil.[960] Ample intakes of the fish-derived long-chain omega-3s have anti-inflammatory effects, and can reduce the tendency of blood platelets to form clots; these effects appear to result from decreased production of certain hormones – prostanoids and leukotrienes – derived from omega-6 fats.[961-965] The anti-inflammatory effects of supplemental EPA/DHA are greater in the context of a diet low in omega-6 oils. Fish oil supplementation can also be modestly beneficial for control of high blood pressure.[966, 967]

The most important benefits of omega-3 fats seem to be mediated by EPA and DHA, the long-chain omega-3s supplied by fish. These fats are not produced by plants, and vegans must generate their own EPA and DHA through conversion of the alpha-linolenic acid which does occur in vegan diets. However, this conversion is notably inefficient in humans, and the health impacts of dietary EPA/DHA are likely to be superior to those of alpha-linolenic acid.[968] Fortunately, vegans can now obtain supplemental DHA generated by algal cultures – albeit this is much more pricy than the omega-3 provided by fish oil. Stearidonic acid, an intermediate in the conversion of alpha-linolenic acid to EPA/DHA, is a minor omega-3 fat that plants can produce, and it is converted to EPA by humans much more efficiently than alpha-linolenic acid.[969] Plants are now being bioengineered to be rich sources of stearidonic acid, and a soy oil rich in this fat is now becoming commercially available;[970] this might prove to be a good option for vegans or for those who oppose the overfishing of our oceans.

Protective Phytochemicals – Soy Isoflavones and Chlorogenic Acid

Many protective phytochemicals can support health by acting as oxidant scavengers, or by boosting cellular expression of antioxidant enzymes; some of these have been discussed above. PhyCB is novel in this regard in that, not only does it act as a scavenger, but, more importantly, it inhibits a prominent source of oxidative stress. But some of the phytochemicals provided by protective foods support health in ways that don't directly pertain to antioxidant defenses.

Soy isoflavones appear to have considerable potential for health promotion. This stems largely from the fact that genistein, one of the most prominent isoflavones in soy, has potent estrogenic activity for the beta form of the estrogen receptor.[971] Estrogen receptors occur in two forms – alpha and beta. Most of the feminizing effects of estrogens are mediated by activation of alpha estrogen receptors. Chronic activation of the alpha estrogen receptor also boosts risks for breast and endometrial cancers. The beta form of the estrogen receptor, in contrast, does not exert feminizing effects, and it actually appears to counteract some of the pro-carcinogenic effects of the alpha estrogen receptor. But it shares some of the beneficial effects of the alpha receptor – promoting health of the bones and vascular system.[972] Also, the beta receptor may help control fibrotic disorders of the liver, kidneys, and heart, and may aid prevention of prostate, breast, and possibly colon cancer.[973-975]

The blood concentrations of free genistein achievable by consuming feasible amounts of soyfoods, or by ingesting recommended doses of soy isoflavone supplements, are high enough to activate the beta estrogen receptor – but too low to meaningfully activate the alpha receptor.[972] (Claims that soy isoflavones function as "weak" estrogens are rubbish – they have strong activity for the beta receptor, but don't meaningfully activate the alpha receptor in physiological concentrations.) That explains why men can eat soy products without becoming feminized – and women can eat soy products without boosting their risk for gynecological cancers. Another prominent soy isoflavone, daidzein, can be converted by gut bacteria to the metabolite equol, which is absorbable, and which shares genistein's property of selectively activating the beta estrogen receptor.[976] Some people achieve this conversion more efficiently than others, and some studies suggest that they derive greater protection from soy isoflavone ingestion than those that are less proficient at equol generation.[976, 977]

There is good reason to suspect that, by selectively stimulating the activity of beta estrogen receptor, regular ingestion of soy isoflavones may aid bone density and vascular health, help prevent a range of fibrotic disorders, and possibly reduce risk for prostate, colon, and breast cancer.[972] (Epidemiological studies in Asia have correlated soy-rich diets with reduced risk for these cancers.) Although some rat studies conclude that soy isoflavones can promote the induction and spread of breast cancer, a careful examination of their protocols reveals that they employ outrageously high dietary levels of isoflavones that would be expected to activate the alpha receptor as well. In fact, recent Asian studies conclude that, in women who already have breast cancer, those whose diets are highest in soy isoflavones tend to have the best prognosis.[978-981] (Ironically, I have spoken with many breast cancer patients whose doctors have counseled them to avoid all soy consumption because soy contains "estrogens".)

Another intriguing phytochemical is **chlorogenic acid**, which is a key component of coffee beans. Heavy regular consumption of either caffeinated or decaffeinated coffee is associated with a dose-dependent decrease in risk for type 2 diabetes (the "maturity-onset" kind linked to overweight).[982-984] People who consume 5-7 cups of coffee a day may reduce their risk for diabetes by up to 50%! Since caffeine is clearly not responsible for this benefit, scientists have looked at other prominent phytochemicals in coffee beans, and the compound chlorogenic acid

has emerged as a likely suspect. Studies in rodents and humans suggest that chlorogenic acid can slow the absorption of dietary carbohydrate, while boosting intestinal production of a hormone that acts on the beta cells of the pancreas (the source of insulin) to promote their proper function.[985, 986] The antidiabetic drug acarbose has similar effects – and has been shown to prevent or delay the onset of diabetes in people at high risk for this disorder. Those who are caffeine-intolerant, or who simply don't like coffee, can obtain chlorogenic acid from low-caffeine extracts of green coffee beans, which are now commercially available as nutraceuticals. A much hyped recent controlled clinical study reports that a high intake of such an extract (1050 mg daily) led to rapid loss of body fat in overweight volunteers;[987] it remains to be seen whether this rather sensational and unanticipated result can be confirmed in future research.

Glucosamine for Healthy Cartilage – and Longer Life?

Glucosamine supplementation has long been recommended for prevention and control of osteoarthritis and osteoarthritic pain. Glucosamine is a biosynthetic precursor for the hyaluronic acid in synovial fluid[988, 989] – which provides viscous lubrication for the joints (intra-articular injections of hyaluronic acid provide rapid relief of osteoarthritic pain) – and of the mucopolysaccharides which are chief components of cartilage. It is not yet certain whether increased synthesis of these compounds contributes to the pain relief and improved preservation of cartilage reported in some clinical trials with glucosamine. Clinical studies with glucosamine as a treatment for osteoarthritis – most have focused on arthritis of the knee – have reached conflicting conclusions, but the majority seem to show some efficacy for pain control; benefit seems to be greatest in those with relatively early osteoarthritis (don't expect a lot if your joint cartilage has already nearly eroded).[990-994] Moreover, two extended controlled studies employing glucosamine sulfate found that loss of knee cartilage was slowed in patients taking the glucosamine.[995-997] And a more recent follow-up of the patients enrolled in this study concluded that over the subsequent 8 years, those who had taken glucosamine for 3 years during the study were less than half as likely to require knee replacement surgery.[998] If this is a genuine effect, it means that glucosamine has a "structure modifying" action, which makes it superior to anti-inflammatory medications such as ibuprofen or celebrex for long-term control of the syndrome and preservation of functional capacity. Moreover, it suggests that glucosamine might even be useful for prevention of osteoarthritis in people who don't yet have it. For people who are dedicated to life-long exercise as a means of staying lean and healthy, this would be a very important benefit indeed.

A number of studies with cultured cartilage cells exposed to pro-inflammatory hormones thought to play a role in the cartilage loss of osteoarthritis, report that glucosamine can exert anti-inflammatory effects that preserve protein synthesis and suppress protein degradation.[999-1003] Whether these studies are clinically relevant is unclear, as the concentrations of glucosamine employed are usually higher than those likely to be achieved during clinical use of this supplement. A similar proviso applies to a recent demonstration that glucosamine can boost the production of hyaluronic acid by cultured synovial tissue.[989]

The inconsistent results of clinical research with glucosamine – the largest, most high profile clinical trial had a negative outcome[1004] - have led to some skepticism regarding its efficacy in medical circles. But the following considerations suggest that glucosamine is more than just a placebo:

- It takes several weeks for the pain-control benefits of glucosamine to become apparent (at least at the standard dose). And it takes several weeks for the benefit to wear off after the supplement is discontinued. This has been reported in numerous clinical trials, and is commonly reported by patients. Placebos tend to work rapidly, and lose their efficacy rapidly after discontinuation.

- Glucosamine supplements were used for years by vets before anyone thought to try them in humans. A placebo effect in a horse or dog?

- Oral administration of glucosamine has prevented loss of cartilage and bone in rabbit models simulating osteoarthritis.[1005, 1006]

- The market for glucosamine is big and getting bigger. Supplements that are phony hypes don't tend to have this staying power.

There may be a simple reason why clinical results with glucosamine have been inconsistent: the recommended dose is too low. The Italian pharmaceutical company Rotta, which introduced its patented glucosamine sulfate preparation years ago, settled on a dose of 1.5 grams daily in its initial clinical trials, which reported positive results; we suspect that financial considerations played a role in choice of this dose schedule. Since then, virtually every clinical study to examine glucosamine has employed the 1.5 gram dose. Yet, during years of recommending glucosamine to people with osteoarthritis, and getting their feedback, the author has gained the strong anecdotal impression that a 3 gram daily dose works better and more quickly than the standard 1.5 gram dose. Indeed, I now recommend an initial intake of 3 grams daily, with the option of cutting back to a lower daily dose once substantial relief has been achieved. If you examine the commercial literature on glucosamine for dogs, you will find doses of over 2 grams daily recommended for 100 pound dogs – with the option of later reducing the dose; the "standard" human dose is recommended for dogs weighing 25-50 pounds!

The fact that essentially all of the sophisticated clinical studies with glucosamine have employed only the "standard" 1.5 gram dose may be indicative of a double standard that accords lesser respect to nutraceuticals. When medical scientists are ready to test a new drug in people, they first do a phase I study in which they test a range of doses to seek the highest dose that is well tolerated and seems to have useful efficacy. They do this because they respect the ability of drugs to improve health, and want to find out how to optimize their usefulness. The fact that it hasn't occurred to researchers to do this with glucosamine may mean that they are less interested in finding out how to use it to best advantage, than to debunk claims for glucosamine which they consider dubious.

We however should note that, in animal studies, excessive concentrations of glucosamine achievable by continuous intravenous administration can have an adverse impact on insulin function.[1007, 1008] This does not appear to be the case with oral doses, and most though not all clinical trials with the "standard" dose show no impact on insulin sensitivity or other vascular risk factors.[1009-1013] Nonetheless, this issue needs to be evaluated in future higher dose trials (if there ever are any!), and people with impaired insulin sensitivity should be aware of this potential problem if they choose to try high doses of glucosamine.

Something else has emerged in the glucosamine literature recently that is particularly intriguing. Epidemiologists at the University of Washington recently followed up a cohort of over 77,000 people over 50 in an effort to determine whether any of the non-vitamin-mineral supplements they took correlated with decreased mortality. Surprisingly, the only positive associations they found were with glucosamine and with chondroitin sulfate (traditionally taken with glucosamine); users of glucosamine were about 18% less likely to die during the follow-up.[1014, 1015] Subsequent analyses also found that glucosamine users were less likely to develop lung or colorectal cancer.[1016] These are just epidemiological associations, and it remains to be seen whether they can be confirmed in other population groups. Nonetheless, the facts that glucosamine exerts a subtle anti-inflammatory effect in osteoarthritis, and that inflammation plays a pathogenic role in so many health disorders, render these findings at least plausible, and worthy of further investigation.

Clearly, there are a great many nutrients, phytochemicals, and herbal extracts that have potential for aiding control of various health disorders. The fact that a given nutraceutical may not have been mentioned in the above discussion should not be construed as evidence that it is of questionable value. I have tried to place emphasis on those agents which have the potential to provide important health benefits to a very high proportion of the population, and whose benefits have been reasonably well documented by medical researchers.

References

(1) Sedlak TW, Snyder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. *Pediatrics* 2004 June;113(6):1776-82.

(2) Lassegue B, Griendling KK. NADPH oxidases: functions and pathologies in the vasculature. *Arterioscler Thromb Vasc Biol* 2010 April;30(4):653-61.

(3) McCarty MF. "Iatrogenic Gilbert syndrome"--a strategy for reducing vascular and cancer risk by increasing plasma unconjugated bilirubin. *Med Hypotheses* 2007;69(5):974-94.

(4) McCarty MF, Barroso-Aranda J, Contreras F. A two-phase strategy for treatment of oxidant-dependent cancers. *Med Hypotheses* 2007;69(3):489-96.

(5) Jaimes EA, DeMaster EG, Tian RX, Raij L. Stable compounds of cigarette smoke induce endothelial superoxide anion production via NADPH oxidase activation. *Arterioscler Thromb Vasc Biol* 2004 June;24(6):1031-6.

(6) Orosz Z, Csiszar A, Labinskyy N et al. Cigarette smoke-induced proinflammatory alterations in the endothelial phenotype: role of NAD(P)H oxidase activation. *Am J Physiol Heart Circ Physiol* 2007 January;292(1):H130-H139.

(7) Shih RH, Lee IT, Hsieh HL, Kou YR, Yang CM. Cigarette smoke extract induces HO-1 expression in mouse cerebral vascular endothelial cells: involvement of c-Src/NADPH oxidase/PDGFR/JAK2/STAT3 pathway. *J Cell Physiol* 2010 November;225(3):741-50.

(8) Asano H, Horinouchi T, Mai Y et al. Nicotine- and tar-free cigarette smoke induces cell damage through reactive oxygen species newly generated by PKC-dependent activation of NADPH oxidase. *J Pharmacol Sci* 2012;118(2):275-87.

(9) Lanone S, Bloc S, Foresti R et al. Bilirubin decreases nos2 expression via inhibition of NAD(P)H oxidase: implications for protection against endotoxic shock in rats. *FASEB J* 2005 November;19(13):1890-2.

(10) Matsumoto H, Ishikawa K, Itabe H, Maruyama Y. Carbon monoxide and bilirubin from heme oxygenase-1 suppresses reactive oxygen species generation and plasminogen activator inhibitor-1 induction. *Mol Cell Biochem* 2006 October;291(1-2):21-8.

(11) Jiang F, Roberts SJ, Datla S, Dusting GJ. NO modulates NADPH oxidase function via heme oxygenase-1 in human endothelial cells. *Hypertension* 2006 November;48(5):950-7.

(12) Datla SR, Dusting GJ, Mori TA, Taylor CJ, Croft KD, Jiang F. Induction of heme oxygenase-1 in vivo suppresses NADPH oxidase derived oxidative stress. *Hypertension* 2007 October;50(4):636-42.

(13) Exner M, Minar E, Wagner O, Schillinger M. The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic Biol Med* 2004 October 15;37(8):1097-104.

(14) Yamaya M, Nakayama K, Ebihara S, Hirai H, Higuchi S, Sasaki H. Relationship between microsatellite polymorphism in the haem oxygenase-1 gene promoter and longevity of the normal Japanese population. *J Med Genet* 2003 February;40(2):146-8.

(15) Schwertner HA, Vitek L. Gilbert syndrome, UGT1A1*28 allele, and cardiovascular disease risk: possible protective effects and therapeutic applications of bilirubin. *Atherosclerosis* 2008 May;198(1):1-11.

(16) Inoguchi T, Sasaki S, Kobayashi K, Takayanagi R, Yamada T. Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes. *JAMA* 2007 September 26;298(12):1398-400.

(17) Inoguchi T, Nawata H. NAD(P)H oxidase activation: a potential target mechanism for diabetic vascular complications, progressive beta-cell dysfunction and metabolic syndrome. *Curr Drug Targets* 2005 June;6(4):495-501.

(18) Schwertner HA, Vitek L. Gilbert syndrome, UGT1A1*28 allele, and cardiovascular disease risk: possible protective effects and therapeutic applications of bilirubin. *Atherosclerosis* 2008 May;198(1):1-11.

(19) Horsfall LJ, Nazareth I, Petersen I. Cardiovascular events as a function of serum bilirubin levels in a large, statin-treated cohort. *Circulation* 2012 November 27;126(22):2556-64.

(20) Horsfall LJ, Rait G, Walters K et al. Serum bilirubin and risk of respiratory disease and death. *JAMA* 2011 February 16;305(7):691-7.

(21) Chan KH, O'Connell RL, Sullivan DR et al. Plasma total bilirubin levels predict amputation events in type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Diabetologia* 2013 January 17.

(22) Cheriyath P, Gorrepati VS, Peters I et al. High Total Bilirubin as a Protective Factor for Diabetes Mellitus: An Analysis of NHANES Data From 1. *J Clin Med Res* 2010 October 11;2(5):201-6.

(23) Fischman D, Valluri A, Gorrepati VS, Murphy ME, Peters I, Cheriyath P. Bilirubin as a Protective Factor for Rheumatoid Arthritis: An NHANES Study of 2. *J Clin Med Res* 2010 December 11;2(6):256-60.

(24) Stender S, Frikke-Schmidt R, Nordestgaard BG, Grande P, Tybjaerg-Hansen A. Genetically elevated bilirubin and risk of ischaemic heart disease: three Mendelian randomization studies and a meta-analysis. *J Intern Med* 2013 January;273(1):59-68.

(25) McArdle PF, Whitcomb BW, Tanner K, Mitchell BD, Shuldiner AR, Parsa A. Association between bilirubin and cardiovascular disease risk factors: using Mendelian randomization to assess causal inference. *BMC Cardiovasc Disord* 2012;12:16.

(26) Rauchschwalbe SK, Zuhlsdorf MT, Schuhly U, Kuhlmann J. Predicting the risk of sporadic elevated bilirubin levels and diagnosing Gilbert's syndrome by genotyping UGT1A1*28 promoter polymorphism. *Int J Clin Pharmacol Ther* 2002 June;40(6):233-40.

(27) Fujii M, Inoguchi T, Sasaki S et al. Bilirubin and biliverdin protect rodents against diabetic nephropathy by downregulating NAD(P)H oxidase. *Kidney Int* 2010 November;78(9):905-19.

(28) Dekker D, Dorresteijn MJ, Pijnenburg M et al. The bilirubin-increasing drug atazanavir improves endothelial function in patients with type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2011 February;31(2):458-63.

(29) Flaig TW, Gustafson DL, Su LJ et al. A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients. *Invest New Drugs* 2007 April;25(2):139-46.

(30) Terry MJ, Maines MD, Lagarias JC. Inactivation of phytochrome- and phycobiliprotein-chromophore precursors by rat liver biliverdin reductase. *J Biol Chem* 1993 December 15;268(35):26099-106.

(31) Romay C, Gonzalez R, Ledon N, Remirez D, Rimbau V. C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci* 2003 June;4(3):207-16.

(32) McCarty MF. Clinical potential of Spirulina as a source of phycocyanobilin. *J Med Food* 2007 December;10(4):566-70.

(33) Chamorro G, Perez-Albiter M, Serrano-Garcia N, Mares-Samano JJ, Rojas P. Spirulina maxima pretreatment partially protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. *Nutr Neurosci* 2006 October;9(5-6):207-12.

(34) Zekry D, Epperson TK, Krause KH. A role for NOX NADPH oxidases in Alzheimer's disease and other types of dementia? *IUBMB Life* 2003 June;55(6):307-13.

(35) Brown GC. Mechanisms of inflammatory neurodegeneration: iNOS and NADPH oxidase. *Biochem Soc Trans* 2007 November;35(Pt 5):1119-21.

(36) Riss J, Decorde K, Sutra T et al. Phycobiliprotein C-Phycocyanin from Spirulina platensis Is Powerfully Responsible for Reducing Oxidative Stress and NADPH

Oxidase Expression Induced by an Atherogenic Diet in Hamsters. *J Agric Food Chem* 2007 September 19;55(19):7962-7.

(37) Zheng J, Inoguchi T, Sasaki S et al. Phycocyanin and Phycocyanobilin from Spirulina Platensis Protect against Diabetic Nephropathy by Inhibiting Oxidative Stress. *Am J Physiol Regul Integr Comp Physiol* 2012 October 31.

(38) Choi BM, Pae HO, Jeong YR, Kim YM, Chung HT. Critical role of heme oxygenase-1 in Foxp3-mediated immune suppression. *Biochem Biophys Res Commun* 2005 February 25;327(4):1066-71.

(39) Xia ZW, Zhong WW, Xu LQ et al. Heme oxygenase-1-mediated CD4+CD25high regulatory T cells suppress allergic airway inflammation. *J Immunol* 2006 November 1;177(9):5936-45.

(40) Xia ZW, Xu LQ, Zhong WW et al. Heme oxygenase-1 attenuates ovalbumin-induced airway inflammation by up-regulation of foxp3 T-regulatory cells, interleukin-10, and membrane-bound transforming growth factor- 1. *Am J Pathol* 2007 December;171(6):1904-14.

(41) Hu AP, Du JM, Li JY, Liu JW. Oridonin promotes CD4+/CD25+ Treg differentiation, modulates Th1/Th2 balance and induces HO-1 in rat splenic lymphocytes. *Inflamm Res* 2008 April;57(4):163-70.

(42) Rocuts F, Zhang X, Yan J et al. Bilirubin promotes de novo generation of T regulatory cells. *Cell Transplant* 2010;19(4):443-51.

(43) Penton-Rol G, Martinez-Sanchez G, Cervantes-Llanos M et al. C-Phycocyanin ameliorates experimental autoimmune encephalomyelitis and induces regulatory T cells. *Int Immunopharmacol* 2010 October 21.

(44) Kraaij MD, Savage ND, van der Kooij SW et al. Induction of regulatory T cells by macrophages is dependent on production of reactive oxygen species. *Proc Natl Acad Sci U S A* 2010 October 12;107(41):17686-91.

(45) Hultqvist M, Olsson LM, Gelderman KA, Holmdahl R. The protective role of ROS in autoimmune disease. *Trends Immunol* 2009 May;30(5):201-8.

(46) Wegiel B, Baty CJ, Gallo D et al. Cell surface biliverdin reductase mediates biliverdin-induced anti-inflammatory effects via phosphatidylinositol 3-kinase and Akt. *J Biol Chem* 2009 August 7;284(32):21369-78.

(47) Liu Y, Zhu B, Wang X et al. Bilirubin as a potent antioxidant suppresses experimental autoimmune encephalomyelitis: implications for the role of oxidative stress in the development of multiple sclerosis. *J Neuroimmunol* 2003 June;139(1-2):27-35.

(48) Liu Y, Li P, Lu J et al. Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis. *J Immunol* 2008 August 1;181(3):1887-97.

(49) Yamashita K, McDaid J, Ollinger R et al. Biliverdin, a natural product of heme catabolism, induces tolerance to cardiac allografts. *FASEB J* 2004 April;18(6):765-7.

(50) Ollinger R, Wang H, Yamashita K et al. Therapeutic applications of bilirubin and biliverdin in transplantation. *Antioxid Redox Signal* 2007 December;9(12):2175-85.

(51) Sarady-Andrews JK, Liu F, Gallo D et al. Biliverdin administration protects against endotoxin-induced acute lung injury in rats. *Am J Physiol Lung Cell Mol Physiol* 2005 December;289(6):L1131-L1137.

(52) Romay C, Delgado R, Remirez D, Gonzalez R, Rojas A. Effects of phycocyanin extract on tumor necrosis factor-alpha and nitrite levels in serum of mice treated with endotoxin. *Arzneimittelforschung* 2001 September;51(9):733-6.

(53) Salazar M, Martinez E, Madrigal E, Ruiz LE, Chamorro GA. Subchronic toxicity study in mice fed Spirulina maxima. *J Ethnopharmacol* 1998 October;62(3):235-41.

(54) Chamorro GA, Herrera G, Salazar M, Salazar S, Ulloa V. Short-term toxicity study of Spirulina in F3b generation rats. *J Toxicol Clin Exp* 1988 May;8(3):163-7.

(55) Salazar M, Chamorro GA, Salazar S, Steele CE. Effect of Spirulina maxima consumption on reproduction and peri- and postnatal development in rats. *Food Chem Toxicol* 1996 April;34(4):353-9.

(56) Chamorro G, Salazar M. [Teratogenic study of Spirulina in mice]. *Arch Latinoam Nutr* 1990 March;40(1):86-94.

(57) Raijmakers MT, Dechend R, Poston L. Oxidative stress and preeclampsia: rationale for antioxidant clinical trials. *Hypertension* 2004 October;44(4):374-80.

(58) Guzy RD, Schumacker PT. Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. *Exp Physiol* 2006 September;91(5):807-19.

(59) Robin E, Guzy RD, Loor G et al. Oxidant stress during simulated ischemia primes cardiomyocytes for cell death during reperfusion. *J Biol Chem* 2007 June 29;282(26):19133-43.

(60) Mariappan N, Elks CM, Fink B, Francis J. TNF-induced mitochondrial damage: a link between mitochondrial complex I activity and left ventricular dysfunction. *Free Radic Biol Med* 2009 February 15;46(4):462-70.

(61) Chan SH, Wu KL, Chang AY, Tai MH, Chan JY. Oxidative impairment of mitochondrial electron transport chain complexes in rostral ventrolateral medulla contributes to neurogenic hypertension. *Hypertension* 2009 February;53(2):217-27.

(62) Doughan AK, Harrison DG, Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. *Circ Res* 2008 February 29;102(4):488-96.

(63) Li WG, Miller FJ, Jr., Zhang HJ, Spitz DR, Oberley LW, Weintraub NL. H(2)O(2)-induced O(2) production by a non-phagocytic NAD(P)H oxidase causes oxidant injury. *J Biol Chem* 2001 August 3;276(31):29251-6.

(64) Becker LB, Vanden Hoek TL, Shao ZH, Li CQ, Schumacker PT. Generation of superoxide in cardiomyocytes during ischemia before reperfusion. *Am J Physiol* 1999 December;277(6 Pt 2):H2240-H2246.

(65) Guzy RD, Schumacker PT. Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. *Exp Physiol* 2006 September;91(5):807-19.

(66) Kurashige M, Okimasu E, Inoue M, Utsumi K. Inhibition of oxidative injury of biological membranes by astaxanthin. *Physiol Chem Phys Med NMR* 1990;22(1):27-38.

(67) Palozza P, Krinsky NI. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. *Arch Biochem Biophys* 1992 September;297(2):291-5.

(68) Miki W. Recent advances in carotenoid studies--metabolisms and bioactivities. *J Nutr Sci Vitaminol (Tokyo)* 1992;Spec No:469-72.

(69) Naguib YM. Antioxidant activities of astaxanthin and related carotenoids. *J Agric Food Chem* 2000 April;48(4):1150-4.

(70) Lorenz RT, Cysewski GR. Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends Biotechnol* 2000 April;18(4):160-7.

(71) Guerin M, Huntley ME, Olaizola M. Haematococcus astaxanthin: applications for human health and nutrition. *Trends Biotechnol* 2003 May;21(5):210-6.

(72) Chen Y, Li D, Lu W, Xing J, Hui B, Han Y. Screening and characterization of astaxanthin-hyperproducing mutants of Haematococcus pluvialis. *Biotechnol Lett* 2003 April;25(7):527-9.

(73) Di MP, Devasagayam TP, Kaiser S, Sies H. Carotenoids, tocopherols and thiols as biological singlet molecular oxygen quenchers. *Biochem Soc Trans* 1990 December;18(6):1054-6.

(74) Cort A, Ozturk N, Akpinar D et al. Suppressive effect of astaxanthin on retinal injury induced by elevated intraocular pressure. *Regul Toxicol Pharmacol* 2010 October;58(1):121-30.

(75) Nakajima Y, Inokuchi Y, Shimazawa M, Otsubo K, Ishibashi T, Hara H. Astaxanthin, a dietary carotenoid, protects retinal cells against oxidative stress in-vitro and in mice in-vivo. *J Pharm Pharmacol* 2008 October;60(10):1365-74.

(76) Manabe E, Handa O, Naito Y et al. Astaxanthin protects mesangial cells from hyperglycemia-induced oxidative signaling. *J Cell Biochem* 2008 April 15;103(6):1925-37.

(77) Liu X, Osawa T. Astaxanthin protects neuronal cells against oxidative damage and is a potent candidate for brain food. *Forum Nutr* 2009;61:129-35.

(78) Liu X, Shibata T, Hisaka S, Osawa T. Astaxanthin inhibits reactive oxygen species-mediated cellular toxicity in dopaminergic SH-SY5Y cells via mitochondria-targeted protective mechanism. *Brain Res* 2009 February 13;1254:18-27.

(79) Wolf AM, Asoh S, Hiranuma H et al. Astaxanthin protects mitochondrial redox state and functional integrity against oxidative stress. *J Nutr Biochem* 2010 May;21(5):381-9.

(80) Lauver DA, Lockwood SF, Lucchesi BR. Disodium Disuccinate Astaxanthin (Cardax) attenuates complement activation and reduces myocardial injury following ischemia/reperfusion. *J Pharmacol Exp Ther* 2005 August;314(2):686-92.

(81) Gross GJ, Lockwood SF. Cardioprotection and myocardial salvage by a disodium disuccinate astaxanthin derivative (Cardax). *Life Sci* 2004 May 28;75(2):215-24.

(82) Pashkow FJ, Watumull DG, Campbell CL. Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease. *Am J Cardiol* 2008 May 22;101(10A):58D-68D.

(83) Shen H, Kuo CC, Chou J et al. Astaxanthin reduces ischemic brain injury in adult rats. *FASEB J* 2009 June;23(6):1958-68.

(84) Curek GD, Cort A, Yucel G et al. Effect of astaxanthin on hepatocellular injury following ischemia/reperfusion. *Toxicology* 2010 January 12;267(1-3):147-53.

(85) Lee DH, Kim CS, Lee YJ. Astaxanthin protects against MPTP/MPP+-induced mitochondrial dysfunction and ROS production in vivo and in vitro. *Food Chem Toxicol* 2010 November 4.

(86) Naito Y, Uchiyama K, Aoi W et al. Prevention of diabetic nephropathy by treatment with astaxanthin in diabetic db/db mice. *Biofactors* 2004;20(1):49-59.

(87) Uchiyama K, Naito Y, Hasegawa G, Nakamura N, Takahashi J, Yoshikawa T. Astaxanthin protects beta-cells against glucose toxicity in diabetic db/db mice. *Redox Rep* 2002;7(5):290-3.

(88) Augusti PR, Conterato GM, Somacal S et al. Astaxanthin reduces oxidative stress, but not aortic damage in atherosclerotic rabbits. *J Cardiovasc Pharmacol Ther* 2009 December;14(4):314-22.

(89) Riss J, Decorde K, Sutra T et al. Phycobiliprotein C-phycocyanin from Spirulina platensis is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. *J Agric Food Chem* 2007 September 19;55(19):7962-7.

(90) Ikeuchi M, Koyama T, Takahashi J, Yazawa K. Effects of astaxanthin supplementation on exercise-induced fatigue in mice. *Biol Pharm Bull* 2006 October;29(10):2106-10.

(91) Aoi W, Naito Y, Takanami Y et al. Astaxanthin improves muscle lipid metabolism in exercise via inhibitory effect of oxidative CPT I modification. *Biochem Biophys Res Commun* 2008 February 22;366(4):892-7.

(92) Ikeuchi M, Koyama T, Takahashi J, Yazawa K. Effects of astaxanthin in obese mice fed a high-fat diet. *Biosci Biotechnol Biochem* 2007 April;71(4):893-9.

(93) Kalafati M, Jamurtas AZ, Nikolaidis MG et al. Ergogenic and antioxidant effects of spirulina supplementation in humans. *Med Sci Sports Exerc* 2010 January;42(1):142-51.

(94) Pessayre D. Role of mitochondria in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2007 June;22 Suppl 1:S20-S27.

(95) Serviddio G, Bellanti F, Sastre J, Vendemiale G, Altomare E. Targeting mitochondria: a new promising approach for the treatment of liver diseases. *Curr Med Chem* 2010;17(22):2325-37.

(96) Rector RS, Thyfault JP, Uptergrove GM et al. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. *J Hepatol* 2010 May;52(5):727-36.

(97) Yoshida H, Yanai H, Ito K et al. Administration of natural astaxanthin increases serum HDL-cholesterol and adiponectin in subjects with mild hyperlipidemia. *Atherosclerosis* 2010 April;209(2):520-3.

(98) Satoh A, Tsuji S, Okada Y et al. Preliminary Clinical Evaluation of Toxicity and Efficacy of A New Astaxanthin-rich Haematococcus pluvialis Extract. *J Clin Biochem Nutr* 2009 May;44(3):280-4.

(99) Uchiyama A, Okada Y. Clinical efficacy of astaxanthin-containing Haematococcus pluvialis extract for the volunteers at risk for metabolic syndrome. *J Clin Biochem Nutr* 2008;43, Suppl 1:38-43.

(100) Furukawa S, Fujita T, Shimabukuro M et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004 December;114(12):1752-61.

(101) Talior I, Tennenbaum T, Kuroki T, Eldar-Finkelman H. PKC-delta-dependent activation of oxidative stress in adipocytes of obese and insulin-resistant mice: role for NADPH oxidase. *Am J Physiol Endocrinol Metab* 2005 February;288(2):E405-E411.

(102) Lee EH, Park JE, Choi YJ, Huh KB, Kim WY. A randomized study to establish the effects of spirulina in type 2 diabetes mellitus patients. *Nutr Res Pract* 2008;2(4):295-300.

(103) Comhaire FH, El GY, Mahmoud A, Eertmans F, Schoonjans F. Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial. *Asian J Androl* 2005 September;7(3):257-62.

(104) Schopfer F, Riobo N, Carreras MC et al. Oxidation of ubiquinol by peroxynitrite: implications for protection of mitochondria against nitrosative damage. *Biochem J* 2000 July 1;349(Pt 1):35-42.

(105) Radi R, Cassina A, Hodara R. Nitric oxide and peroxynitrite interactions with mitochondria. *Biol Chem* 2002 March;383(3-4):401-9.

(106) Valdez LB, Alvarez S, Arnaiz SL et al. Reactions of peroxynitrite in the mitochondrial matrix. *Free Radic Biol Med* 2000 August;29(3-4):349-56.

(107) Yamagami T, Shibata N, Folkers K. Bioenergetics in clinical medicine. VIII. Adminstration of coenzyme Q10 to patients with essential hypertension. *Res Commun Chem Pathol Pharmacol* 1976 August;14(4):721-7.

(108) Hansen IL, Iwamoto Y, Kishi T, Folkers K, Thompson LE. Bioenergetics in clinical medicine. IX. Gingival and leucocytic deficiencies of coenzyme Q10 in patients with periodontal disease. *Res Commun Chem Pathol Pharmacol* 1976 August;14(4):729-38.

(109) Keogh A, Fenton S, Leslie C et al. Randomised double-blind, placebo-controlled trial of coenzyme Q, therapy in class II and III systolic heart failure. *Heart Lung Circ* 2003;12(3):135-41.

(110) Rosenfeldt F, Hilton D, Pepe S, Krum H. Systematic review of effect of coenzyme Q10 in physical exercise, hypertension and heart failure. *Biofactors* 2003;18(1-4):91-100.

(111) Soja AM, Mortensen SA. Treatment of congestive heart failure with coenzyme Q10 illuminated by meta-analyses of clinical trials. *Mol Aspects Med* 1997;18 Suppl:S159-S168.

(112) Sander S, Coleman CI, Patel AA, Kluger J, White CM. The impact of coenzyme Q10 on systolic function in patients with chronic heart failure. *J Card Fail* 2006 August;12(6):464-72.

(113) Molyneux SL, Florkowski CM, George PM et al. Coenzyme Q10: an independent predictor of mortality in chronic heart failure. *J Am Coll Cardiol* 2008 October 28;52(18):1435-41.

(114) Conklin KA. Coenzyme q10 for prevention of anthracycline-induced cardiotoxicity. *Integr Cancer Ther* 2005 June;4(2):110-30.

(115) Rosenfeldt FL, Haas SJ, Krum H et al. Coenzyme Q10 in the treatment of hypertension: a meta-analysis of the clinical trials. *J Hum Hypertens* 2007 April;21(4):297-306.

(116) Zimmerman MC, Lazartigues E, Sharma RV, Davisson RL. Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system. *Circ Res* 2004 July 23;95(2):210-6.

(117) Tai MH, Wang LL, Wu KL, Chan JY. Increased superoxide anion in rostral ventrolateral medulla contributes to hypertension in spontaneously hypertensive rats via interactions with nitric oxide. *Free Radic Biol Med* 2005 February 15;38(4):450-62.

(118) Peterson JR, Sharma RV, Davisson RL. Reactive oxygen species in the neuropathogenesis of hypertension. *Curr Hypertens Rep* 2006 June;8(3):232-41.

(119) Nagae A, Fujita M, Kawarazaki H, Matsui H, Ando K, Fujita T. Sympathoexcitation by Oxidative Stress in the Brain Mediates Arterial Pressure Elevation in Obesity-Induced Hypertension. *Circulation* 2009 February 9.

(120) Fujita M, Ando K, Nagae A, Fujita T. Sympathoexcitation by oxidative stress in the brain mediates arterial pressure elevation in salt-sensitive hypertension. *Hypertension* 2007 August;50(2):360-7.

(121) Watts GF, Playford DA, Croft KD, Ward NC, Mori TA, Burke V. Coenzyme Q(10) improves endothelial dysfunction of the brachial artery in Type II diabetes mellitus. *Diabetologia* 2002 March;45(3):420-6.

(122) Hodgson JM, Watts GF. Can coenzyme Q10 improve vascular function and blood pressure? Potential for effective therapeutic reduction in vascular oxidative stress. *Biofactors* 2003;18(1-4):129-36.

(123) Tiano L, Belardinelli R, Carnevali P, Principi F, Seddaiu G, Littarru GP. Effect of coenzyme Q10 administration on endothelial function and extracellular superoxide dismutase in patients with ischaemic heart disease: a double-blind, randomized controlled study. *Eur Heart J* 2007 September;28(18):2249-55.

(124) Yokoyama H, Lingle DM, Crestanello JA et al. Coenzyme Q10 protects coronary endothelial function from ischemia reperfusion injury via an antioxidant effect. *Surgery* 1996 August;120(2):189-96.

(125) Maulik N, Yoshida T, Engelman RM, Bagchi D, Otani H, Das DK. Dietary coenzyme Q(10) supplement renders swine hearts resistant to ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2000 April;278(4):H1084-H1090.

(126) Crestanello JA, Doliba NM, Doliba NM et al. Effect of coenzyme Q10 supplementation on mitochondrial function after myocardial ischemia reperfusion. *J Surg Res* 2002 February;102(2):221-8.

(127) Liu B, Tewari AK, Zhang L et al. Proteomic analysis of protein tyrosine nitration after ischemia reperfusion injury: Mitochondria as the major target. *Biochim Biophys Acta* 2009 March;1794(3):476-85.

(128) Cooke M, Iosia M, Buford T et al. Effects of acute and 14-day coenzyme Q10 supplementation on exercise performance in both trained and untrained individuals. *J Int Soc Sports Nutr* 2008;5:8.

(129) Kon M, Tanabe K, Akimoto T et al. Reducing exercise-induced muscular injury in kendo athletes with supplementation of coenzyme Q10. *Br J Nutr* 2008 October;100(4):903-9.

(130) Mizuno K, Tanaka M, Nozaki S et al. Antifatigue effects of coenzyme Q10 during physical fatigue. *Nutrition* 2008 April;24(4):293-9.

(131) Kon M, Kimura F, Akimoto T et al. Effect of Coenzyme Q10 supplementation on exercise-induced muscular injury of rats. *Exerc Immunol Rev* 2007;13:76-88.

(132) Beal MF. Mitochondrial dysfunction and oxidative damage in Alzheimer's and Parkinson's diseases and coenzyme Q10 as a potential treatment. *J Bioenerg Biomembr* 2004 August;36(4):381-6.

(133) Young AJ, Johnson S, Steffens DC, Doraiswamy PM. Coenzyme Q10: a review of its promise as a neuroprotectant. *CNS Spectr* 2007 January;12(1):62-8.

(134) Cleren C, Yang L, Lorenzo B et al. Therapeutic effects of coenzyme Q10 (CoQ10) and reduced CoQ10 in the MPTP model of Parkinsonism. *J Neurochem* 2008 March;104(6):1613-21.

(135) Abdin AA, Hamouda HE. Mechanism of the neuroprotective role of coenzyme Q10 with or without L-dopa in rotenone-induced parkinsonism. *Neuropharmacology* 2008 December;55(8):1340-6.

(136) Shults CW, Oakes D, Kieburtz K et al. Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline. *Arch Neurol* 2002 October;59(10):1541-50.

(137) Muller T, Buttner T, Gholipour AF, Kuhn W. Coenzyme Q10 supplementation provides mild symptomatic benefit in patients with Parkinson's disease. *Neurosci Lett* 2003 May 8;341(3):201-4.

(138) Shults CW, Haas R. Clinical trials of coenzyme Q10 in neurological disorders. *Biofactors* 2005;25(1-4):117-26.

(139) Storch A, Jost WH, Vieregge P et al. Randomized, double-blind, placebo-controlled trial on symptomatic effects of coenzyme Q(10) in Parkinson disease. *Arch Neurol* 2007 July;64(7):938-44.

(140) Singh RB, Niaz MA, Rastogi SS, Shukla PK, Thakur AS. Effect of hydrosoluble coenzyme Q10 on blood pressures and insulin resistance in hypertensive patients with coronary artery disease. *J Hum Hypertens* 1999 March;13(3):203-8.

(141) Kunitomo M, Yamaguchi Y, Kagota S, Otsubo K. Beneficial effect of coenzyme Q10 on increased oxidative and nitrative stress and inflammation and individual metabolic components developing in a rat model of metabolic syndrome. *J Pharmacol Sci* 2008 June;107(2):128-37.

(142) Hodgson JM, Watts GF, Playford DA, Burke V, Croft KD. Coenzyme Q10 improves blood pressure and glycaemic control: a controlled trial in subjects with type 2 diabetes. *Eur J Clin Nutr* 2002 November;56(11):1137-42.

(143) Eriksson JG, Forsen TJ, Mortensen SA, Rohde M. The effect of coenzyme Q10 administration on metabolic control in patients with type 2 diabetes mellitus. *Biofactors* 1999;9(2-4):315-8.

(144) Langsjoen PH, Langsjoen JO, Langsjoen AM, Lucas LA. Treatment of statin adverse effects with supplemental Coenzyme Q10 and statin drug discontinuation. *Biofactors* 2005;25(1-4):147-52.

(145) Schaars CF, Stalenhoef AF. Effects of ubiquinone (coenzyme Q10) on myopathy in statin users. *Curr Opin Lipidol* 2008 December;19(6):553-7.

(146) Young JM, Florkowski CM, Molyneux SL et al. Effect of coenzyme Q(10) supplementation on simvastatin-induced myalgia. *Am J Cardiol* 2007 November 1;100(9):1400-3.

(147) Ferrante KL, Shefner J, Zhang H et al. Tolerance of high-dose (3,000 mg/day) coenzyme Q10 in ALS. *Neurology* 2005 December 13;65(11):1834-6.

(148) Chopra RK, Goldman R, Sinatra ST, Bhagavan HN. Relative bioavailability of coenzyme Q10 formulations in human subjects. *Int J Vitam Nutr Res* 1998;68(2):109-13.

(149) Singh RB, Niaz MA, Kumar A, Sindberg CD, Moesgaard S, Littarru GP. Effect on absorption and oxidative stress of different oral Coenzyme Q10 dosages and intake strategy in healthy men. *Biofactors* 2005;25(1-4):219-24.

(150) Bhagavan HN, Chopra RK. Plasma coenzyme Q10 response to oral ingestion of coenzyme Q10 formulations. *Mitochondrion* 2007 June;7 Suppl:S78-S88.

(151) Alvarez B, Radi R. Peroxynitrite reactivity with amino acids and proteins. *Amino Acids* 2003 December;25(3-4):295-311.

(152) Sawa T, Ohshima H. Nitrative DNA damage in inflammation and its possible role in carcinogenesis. *Nitric Oxide* 2006 March;14(2):91-100.

(153) Pacher P, Szabo C. Role of the peroxynitrite-poly(ADP-ribose) polymerase pathway in human disease. *Am J Pathol* 2008 July;173(1):2-13.

(154) Szabo C, Ischiropoulos H, Radi R. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat Rev Drug Discov* 2007 August;6(8):662-80.

(155) Guven A, Uysal B, Akgul O et al. Scavenging of peroxynitrite reduces renal ischemia/reperfusion injury. *Ren Fail* 2008;30(7):747-54.

(156) Jiao X, Gao E, Yuang Y et al. INO-4885, a Peroxynitrite Decomposition Catalyst, Protects the Heart against Reperfusion Injury in Mice. *J Pharmacol Exp Ther* 2008 November 25.

(157) Evgenov OV, Liaudet L. Role of nitrosative stress and activation of poly(ADP-ribose) polymerase-1 in cardiovascular failure associated with septic and hemorrhagic shock. *Curr Vasc Pharmacol* 2005 July;3(3):293-9.

(158) Snook JH, Li J, Helmke BP, Guilford WH. Peroxynitrite inhibits myofibrillar protein function in an in vitro assay of motility. *Free Radic Biol Med* 2008 January 1;44(1):14-23.

(159) Milstien S, Katusic Z. Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. *Biochem Biophys Res Commun* 1999 October 5;263(3):681-4.

(160) Zou M, Martin C, Ullrich V. Tyrosine nitration as a mechanism of selective inactivation of prostacyclin synthase by peroxynitrite. *Biol Chem* 1997 July;378(7):707-13.

(161) Nishino T, Okamoto K, Eger BT, Pai EF, Nishino T. Mammalian xanthine oxidoreductase - mechanism of transition from xanthine dehydrogenase to xanthine oxidase. *FEBS J* 2008 July;275(13):3278-89.

(162) Squadrito GL, Cueto R, Splenser AE et al. Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid. *Arch Biochem Biophys* 2000 April 15;376(2):333-7.

(163) Oster KA, Ross DJ, Dawkins HHR. *The XO Factor*. New York: Park City Press; 1983.

(164) Ross DJ, Oster KA. Digestion and absorption of bovine milk xanthine oxidase. *J Nutr* 1978 June;108(6):1039-42.

(165) Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. *Lancet* 2010 June 19;375(9732):2161-7.

(166) Boss GR, Ragsdale RA, Zettner A, Seegmiller JE. Failure of folic acid (pteroylglutamic acid) to affect hyperuricemia. *J Lab Clin Med* 1980 November;96(5):783-9.

(167) Oster KA. Atherosclerosis treated with folic acid. *FASEB J* 1981;40.

(168) Oster KA. A cardiologist considers psoriasis. *Cutis* 1977 July;20(1):39-40, 45.

(169) Heinloth A, Heermeier K, Raff U, Wanner C, Galle J. Stimulation of NADPH oxidase by oxidized low-density lipoprotein induces proliferation of human vascular endothelial cells. *J Am Soc Nephrol* 2000 October;11(10):1819-25.

(170) Cai H, Griendling KK, Harrison DG. The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. *Trends Pharmacol Sci* 2003 September;24(9):471-8.

(171) McCarty MF. Oster rediscovered--mega-dose folate for symptomatic atherosclerosis. *Med Hypotheses* 2007;69(2):325-32.

(172) Verhaar MC, Wever RM, Kastelein JJ, van DT, Koomans HA, Rabelink TJ. 5-methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolemia. *Circulation* 1998 January 27;97(3):237-41.

(173) Stroes ES, van Faassen EE, Yo M et al. Folic acid reverts dysfunction of endothelial nitric oxide synthase. *Circ Res* 2000 June 9;86(11):1129-34.

(174) Hyndman ME, Verma S, Rosenfeld RJ, Anderson TJ, Parsons HG. Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function. *Am J Physiol Heart Circ Physiol* 2002 June;282(6):H2167-H2172.

(175) Antoniades C, Shirodaria C, Warrick N et al. 5-methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. *Circulation* 2006 September 12;114(11):1193-201.

(176) Rezk BM, Haenen GR, Van d, V, Bast A. Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore. *FEBS Lett* 2003 December 18;555(3):601-5.

(177) Spinella MJ, Brigle KE, Sierra EE, Goldman ID. Distinguishing between folate receptor-alpha-mediated transport and reduced folate carrier-mediated transport in L1210 leukemia cells. *J Biol Chem* 1995 April 7;270(14):7842-9.

(178) Obeid R, Kirsch SH, Kasoha M, Eckert R, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in plasma after folic acid treatment in older adults. *Metabolism* 2011 May;60(5):673-80.

(179) Tawakol A, Migrino RQ, Aziz KS et al. High-dose folic acid acutely improves coronary vasodilator function in patients with coronary artery disease. *J Am Coll Cardiol* 2005 May 17;45(10):1580-4.

(180) Moens AL, Claeys MJ, Wuyts FL et al. Effect of folic acid on endothelial function following acute myocardial infarction. *Am J Cardiol* 2007 February 15;99(4):476-81.

(181) Moens AL, Champion HC, Claeys MJ et al. High-Dose Folic Acid Pretreatment Blunts Cardiac Dysfunction During Ischemia Coupled to Maintenance of High-Energy Phosphates and Reduces Postreperfusion Injury. *Circulation* 2008 March 24.

(182) Tian R, Ingwall JS. How does folic acid cure heart attacks? *Circulation* 2008 April 8;117(14):1772-4.

(183) Cagnacci A, Cannoletta M, Volpe A. High-dose short-term folate administration modifies ambulatory blood pressure in postmenopausal women. A placebo-controlled study. *Eur J Clin Nutr* 2009 July 15.

(184) Dong F, Zhang X, Li SY et al. Possible involvement of NADPH oxidase and JNK in homocysteine-induced oxidative stress and apoptosis in human umbilical vein endothelial cells. *Cardiovasc Toxicol* 2005;5(1):9-20.

(185) Wierzbicki AS. Homocysteine and cardiovascular disease: a review of the evidence. *Diab Vasc Dis Res* 2007 June;4(2):143-50.

(186) McCarty MF, Barroso-Aranda J, Contreras F. High-dose folate and dietary purines promote scavenging of peroxynitrite-derived radicals - clinical potential in inflammatory disorders. *Medical Hypotheses* 2009;accepted for publication.

(187) Sarna LK, Wu N, Wang P, Hwang SY, Siow YL, O K. Folic acid supplementation attenuates high fat diet induced hepatic oxidative stress via regulation of NADPH oxidase. *Can J Physiol Pharmacol* 2012 February;90(2):155-65.

(188) Lee SJ, Kang MH, Min H. Folic acid supplementation reduces oxidative stress and hepatic toxicity in rats treated chronically with ethanol. *Nutr Res Pract* 2011 December;5(6):520-6.

(189) Kim YI. Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr* 2004 November;80(5):1123-8.

(190) Bistulfi G, Vandette E, Matsui S, Smiraglia DJ. Mild folate deficiency induces genetic and epigenetic instability and phenotype changes in prostate cancer cells. *BMC Biol* 2010;8:6.

(191) Wien TN, Pike E, Wisloff T, Staff A, Smeland S, Klemp M. Cancer risk with folic acid supplements: a systematic review and meta-analysis. *BMJ Open* 2012;2(1):e000653.

(192) Bistulfi G, Diegelman P, Foster BA, Kramer DL, Porter CW, Smiraglia DJ. Polyamine biosynthesis impacts cellular folate requirements necessary to maintain S-adenosylmethionine and nucleotide pools. *FASEB J* 2009 September;23(9):2888-97.

(193) Bistulfi G, Foster BA, Karasik E et al. Dietary folate deficiency blocks prostate cancer progression in the TRAMP model. *Cancer Prev Res (Phila)* 2011 November;4(11):1825-34.

(194) Giovannucci E, Stampfer MJ, Colditz GA et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. *Ann Intern Med* 1998 October 1;129(7):517-24.

(195) Jardine LF, Ingram LC, Bleyer WA. Intrathecal leucovorin after intrathecal methotrexate overdose. *J Pediatr Hematol Oncol* 1996 August;18(3):302-4.

(196) Weisskopf MG, O'Reilly E, Chen H, Schwarzschild MA, Ascherio A. Plasma urate and risk of Parkinson's disease. *Am J Epidemiol* 2007 September 1;166(5):561-7.

(197) Gao X, Chen H, Choi HK, Curhan G, Schwarzschild MA, Ascherio A. Diet, urate, and Parkinson's disease risk in men. *Am J Epidemiol* 2008 April 1;167(7):831-8.

(198) Chen H, O'Reilly E, McCullough ML et al. Consumption of dairy products and risk of Parkinson's disease. *Am J Epidemiol* 2007 May 1;165(9):998-1006.

(199) Garrel DR, Verdy M, PetitClerc C, Martin C, Brule D, Hamet P. Milk- and soy-protein ingestion: acute effect on serum uric acid concentration. *Am J Clin Nutr* 1991 March;53(3):665-9.

(200) Choi HK, Atkinson K, Karlson EW, Willett W, Curhan G. Purine-rich foods, dairy and protein intake, and the risk of gout in men. *N Engl J Med* 2004 March 11;350(11):1093-103.

(201) Spitsin S, Koprowski H. Role of uric acid in multiple sclerosis. *Curr Top Microbiol Immunol* 2008;318:325-42.

(202) Scott GS, Spitsin SV, Kean RB, Mikheeva T, Koprowski H, Hooper DC. Therapeutic intervention in experimental allergic encephalomyelitis by administration of uric acid precursors. *Proc Natl Acad Sci U S A* 2002 December 10;99(25):16303-8.

(203) Spitsin S, Hooper DC, Leist T, Streletz LJ, Mikheeva T, Koprowskil H. Inactivation of peroxynitrite in multiple sclerosis patients after oral administration of inosine may suggest possible approaches to therapy of the disease. *Mult Scler* 2001 October;7(5):313-9.

(204) Toncev G. Therapeutic value of serum uric acid levels increasing in the treatment of multiple sclerosis. *Vojnosanit Pregl* 2006 October;63(10):879-82.

(205) Markowitz CE, Spitsin S, Zimmerman V et al. The treatment of multiple sclerosis with inosine. *J Altern Complement Med* 2009 June;15(6):619-25.

(206) Gonsette RE, Sindic C, D'hooghe MB et al. Boosting endogenous neuroprotection in multiple sclerosis: the ASsociation of Inosine and Interferon beta in relapsing-remitting Multiple Sclerosis (ASIIMS) trial. *Mult Scler* 2010 April;16(4):455-62.

(207) Reyes AJ. Potassium-retaining diuretics and incident Alzheimer disease. *Arch Neurol* 2006 October;63(10):1507.

(208) Sautin YY, Nakagawa T, Zharikov S, Johnson RJ. Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress. *Am J Physiol Cell Physiol* 2007 August;293(2):C584-C596.

(209) Chao HH, Liu JC, Lin JW, Chen CH, Wu CH, Cheng TH. Uric acid stimulates endothelin-1 gene expression associated with NADPH oxidase in human aortic smooth muscle cells. *Acta Pharmacol Sin* 2008 November;29(11):1301-12.

(210) Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med* 2008 October 23;359(17):1811-21.

(211) Puig JG, Martinez MA. Hyperuricemia, gout and the metabolic syndrome. *Curr Opin Rheumatol* 2008 March;20(2):187-91.

(212) Muscelli E, Natali A, Bianchi S et al. Effect of insulin on renal sodium and uric acid handling in essential hypertension. *Am J Hypertens* 1996 August;9(8):746-52.

(213) Waring WS, Adwani SH, Breukels O, Webb DJ, Maxwell SR. Hyperuricaemia does not impair cardiovascular function in healthy adults. *Heart* 2004 February;90(2):155-9.

(214) Waring WS, McKnight JA, Webb DJ, Maxwell SR. Lowering serum urate does not improve endothelial function in patients with type 2 diabetes. *Diabetologia* 2007 December;50(12):2572-9.

(215) Waring WS, McKnight JA, Webb DJ, Maxwell SR. Uric acid restores endothelial function in patients with type 1 diabetes and regular smokers. *Diabetes* 2006 November;55(11):3127-32.

(216) Spitsin S, Markowitz CE, Zimmerman V, Koprowski H, Hooper DC. Modulation of serum uric acid levels by inosine in patients with multiple sclerosis does not affect blood pressure. *J Hum Hypertens* 2010 May;24(5):359-62.

(217) Frank BA. *Nucleic Acid Therapy in Aging and Degenerative Disease*. New York: Psychological Library; 1968.

(218) Kohr MJ, Wang H, Wheeler DG, Velayutham M, Zweier JL, Ziolo MT. Targeting of phospholamban by peroxynitrite decreases beta-adrenergic stimulation in cardiomyocytes. *Cardiovasc Res* 2008 January 15;77(2):353-61.

(219) Nishino T, Okamoto K, Eger BT, Pai EF, Nishino T. Mammalian xanthine oxidoreductase - mechanism of transition from xanthine dehydrogenase to xanthine oxidase. *FEBS J* 2008 July;275(13):3278-89.

(220) Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. *Lancet* 2010 June 19;375(9732):2161-7.

(221) George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. *Circulation* 2006 December 5;114(23):2508-16.

(222) Augustin AJ, Boker T, Blumenroder SH, Lutz J, Spitznas M. Free radical scavenging and antioxidant activity of allopurinol and oxypurinol in experimental lens-induced uveitis. *Invest Ophthalmol Vis Sci* 1994 October;35(11):3897-904.

(223) Struthers A, Shearer F. Allopurinol: novel indications in cardiovascular disease. *Heart* 2012 November;98(21):1543-5.

(224) Goicoechea M, de Vinuesa SG, Verdalles U et al. Effect of allopurinol in chronic kidney disease progression and cardiovascular risk. *Clin J Am Soc Nephrol* 2010 August;5(8):1388-93.

(225) Kao MP, Ang DS, Gandy SJ et al. Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease. *J Am Soc Nephrol* 2011 July;22(7):1382-9.

(226) Surh YJ, Kundu JK, Na HK. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Med* 2008 October;74(13):1526-39.

(227) Zhang DD, Lo SC, Cross JV, Templeton DJ, Hannink M. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol Cell Biol* 2004 December;24(24):10941-53.

(228) Shay KP, Michels AJ, Li W, Kong AN, Hagen TM. Cap-independent Nrf2 translation is part of a lipoic acid-stimulated detoxification stress response. *Biochim Biophys Acta* 2012 June;1823(6):1102-9.

(229) Li W, Thakor N, Xu EY et al. An internal ribosomal entry site mediates redox-sensitive translation of Nrf2. *Nucleic Acids Res* 2010 January;38(3):778-88.

(230) Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci U S A* 1997 September 16;94(19):10367-72.

(231) Riedl MA, Saxon A, az-Sanchez D. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. *Clin Immunol* 2008 November 21.

(232) Flier J, Van Muiswinkel FL, Jongenelen CA, Drukarch B. The neuroprotective antioxidant alpha-lipoic acid induces detoxication enzymes in cultured astroglial cells. *Free Radic Res* 2002 June;36(6):695-9.

(233) Cao Z, Tsang M, Zhao H, Li Y. Induction of endogenous antioxidants and phase 2 enzymes by alpha-lipoic acid in rat cardiac H9C2 cells: protection against oxidative injury. *Biochem Biophys Res Commun* 2003 October 24;310(3):979-85.

(234) Jia Z, Hallur S, Zhu H, Li Y, Misra HP. Potent upregulation of glutathione and NAD(P)H:quinone oxidoreductase 1 by alpha-lipoic acid in human neuroblastoma SH-SY5Y cells: protection against neurotoxicant-elicited cytotoxicity. *Neurochem Res* 2008 May;33(5):790-800.

(235) Packer L, Tritschler HJ, Wessel K. Neuroprotection by the metabolic antioxidant alpha-lipoic acid. *Free Radic Biol Med* 1997;22(1-2):359-78.

(236) Ziegler D, Ametov A, Barinov A et al. Oral treatment with alpha-lipoic acid improves symptomatic diabetic polyneuropathy: the SYDNEY 2 trial. *Diabetes Care* 2006 November;29(11):2365-70.

(237) Berkson BM. Thioctic acid in treatment of hepatotoxic mushroom (Phalloides) poisoning. *N Engl J Med* 1979 February 15;300(7):371.

(238) Berkson BM. A conservative triple antioxidant approach to the treatment of hepatitis C. Combination of alpha lipoic acid (thioctic acid), silymarin, and selenium: three case histories. *Med Klin (Munich)* 1999 October 15;94 Suppl 3:84-9.

(239) Smith AR, Shenvi SV, Widlansky M, Suh JH, Hagen TM. Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Curr Med Chem* 2004 May;11(9):1135-46.

(240) Yi X, Maeda N. alpha-Lipoic acid prevents the increase in atherosclerosis induced by diabetes in apolipoprotein E-deficient mice fed high-fat/low-cholesterol diet. *Diabetes* 2006 August;55(8):2238-44.

(241) Panigrahi M, Sadguna Y, Shivakumar BR et al. alpha-Lipoic acid protects against reperfusion injury following cerebral ischemia in rats. *Brain Res* 1996 April 22;717(1-2):184-8.

(242) Sehirli O, Sener E, Cetinel S, Yuksel M, Gedik N, Sener G. Alpha-lipoic acid protects against renal ischaemia-reperfusion injury in rats. *Clin Exp Pharmacol Physiol* 2008 March;35(3):249-55.

(243) Johnsen-Soriano S, Garcia-Pous M, Arnal E et al. Early lipoic acid intake protects retina of diabetic mice. *Free Radic Res* 2008 July;42(7):613-7.

(244) Kojima M, Sun L, Hata I, Sakamoto Y, Sasaki H, Sasaki K. Efficacy of alpha-lipoic acid against diabetic cataract in rat. *Jpn J Ophthalmol* 2007 January;51(1):10-3.

(245) Lateef H, Aslam MN, Stevens MJ, Varani J. Pretreatment of diabetic rats with lipoic acid improves healing of subsequently-induced abrasion wounds. *Arch Dermatol Res* 2005 August;297(2):75-83.

(246) Kowluru RA, Odenbach S. Effect of long-term administration of alpha-lipoic acid on retinal capillary cell death and the development of retinopathy in diabetic rats. *Diabetes* 2004 December;53(12):3233-8.

(247) Baydas G, Donder E, Kiliboz M et al. Neuroprotection by alpha-lipoic acid in streptozotocin-induced diabetes. *Biochemistry (Mosc)* 2004 September;69(9):1001-5.

(248) Melhem MF, Craven PA, DeRubertis FR. Effects of dietary supplementation of alpha-lipoic acid on early glomerular injury in diabetes mellitus. *J Am Soc Nephrol* 2001 January;12(1):124-33.

(249) Vincent HK, Bourguignon CM, Vincent KR, Taylor AG. Effects of alpha-lipoic acid supplementation in peripheral arterial disease: a pilot study. *J Altern Complement Med* 2007 June;13(5):577-84.

(250) Hager K, Kenklies M, McAfoose J, Engel J, Munch G. Alpha-lipoic acid as a new treatment option for Alzheimer's disease--a 48 months follow-up analysis. *J Neural Transm Suppl* 2007;(72):189-93.

(251) Na HK, Surh YJ. Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. *Food Chem Toxicol* 2008 April;46(4):1271-8.

(252) Tomata Y, Kakizaki M, Nakaya N et al. Green tea consumption and the risk of incident functional disability in elderly Japanese: the Ohsaki Cohort 2006 Study. *Am J Clin Nutr* 2012 March;95(3):732-9.

(253) Niu K, Hozawa A, Kuriyama S et al. Green tea consumption is associated with depressive symptoms in the elderly. *Am J Clin Nutr* 2009 December;90(6):1615-22.

(254) Kuriyama S. The relation between green tea consumption and cardiovascular disease as evidenced by epidemiological studies. *J Nutr* 2008 August;138(8):1548S-53S.

(255) Kuriyama S, Shimazu T, Ohmori K et al. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *JAMA* 2006 September 13;296(10):1255-65.

(256) Kuriyama S, Hozawa A, Ohmori K et al. Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project 1. *Am J Clin Nutr* 2006 February;83(2):355-61.

(257) Muraki S, Yamamoto S, Ishibashi H et al. Diet and lifestyle associated with increased bone mineral density: cross-sectional study of Japanese elderly women at an osteoporosis outpatient clinic. *J Orthop Sci* 2007 July;12(4):317-20.

(258) Sasazuki S, Kodama H, Yoshimasu K et al. Relation between green tea consumption and the severity of coronary atherosclerosis among Japanese men and women. *Ann Epidemiol* 2000 August;10(6):401-8.

(259) Yang YC, Lu FH, Wu JS, Wu CH, Chang CJ. The protective effect of habitual tea consumption on hypertension. *Arch Intern Med* 2004 July 26;164(14):1534-40.

(260) Mineharu Y, Koizumi A, Wada Y et al. Coffee, green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. *J Epidemiol Community Health* 2011 March;65(3):230-40.

(261) Liang W, Lee AH, Binns CW, Huang R, Hu D, Zhou Q. Tea consumption and ischemic stroke risk: a case-control study in southern China. *Stroke* 2009 July;40(7):2480-5.

(262) Arab L, Liu W, Elashoff D. Green and black tea consumption and risk of stroke: a meta-analysis. *Stroke* 2009 May;40(5):1786-92.

(263) Tanabe N, Suzuki H, Aizawa Y, Seki N. Consumption of green and roasted teas and the risk of stroke incidence: results from the Tokamachi-Nakasato cohort study in Japan. *Int J Epidemiol* 2008 October;37(5):1030-40.

(264) Suzuki E, Yorifuji T, Takao S et al. Green tea consumption and mortality among Japanese elderly people: the prospective Shizuoka elderly cohort. *Ann Epidemiol* 2009 October;19(10):732-9.

(265) Shen CL, Chyu MC, Yeh JK et al. Effect of green tea and Tai Chi on bone health in postmenopausal osteopenic women: a 6-month randomized placebo-controlled trial. *Osteoporos Int* 2011 July 16.

(266) Pullikotil P, Chen H, Muniyappa R et al. Epigallocatechin gallate induces expression of heme oxygenase-1 in endothelial cells via p38 MAPK and Nrf-2 that suppresses proinflammatory actions of TNF-alpha. *J Nutr Biochem* 2011 November 30.

(267) Oyama J, Maeda T, Kouzuma K et al. Green tea catechins improve human forearm endothelial dysfunction and have antiatherosclerotic effects in smokers. *Circ J* 2010 March;74(3):578-88.

(268) Oyama J, Maeda T, Sasaki M et al. Green tea catechins improve human forearm vascular function and have potent anti-inflammatory and anti-apoptotic effects in smokers. *Intern Med* 2010;49(23):2553-9.

(269) Kim W, Jeong MH, Cho SH et al. Effect of green tea consumption on endothelial function and circulating endothelial progenitor cells in chronic smokers. *Circ J* 2006 August;70(8):1052-7.

(270) Widlansky ME, Hamburg NM, Anter E et al. Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. *J Am Coll Nutr* 2007 April;26(2):95-102.

(271) Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev* 1998 April;7(4):351-4.

(272) Rezai-Zadeh K, Shytle D, Sun N et al. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J Neurosci* 2005 September 21;25(38):8807-14.

(273) Levites Y, Amit T, Mandel S, Youdim MB. Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (-)-epigallocatechin-3-gallate. *FASEB J* 2003 May;17(8):952-4.

(274) Obregon DF, Rezai-Zadeh K, Bai Y et al. ADAM10 activation is required for green tea (-)-epigallocatechin-3-gallate-induced alpha-secretase cleavage of amyloid precursor protein. *J Biol Chem* 2006 June 16;281(24):16419-27.

(275) Rezai-Zadeh K, Arendash GW, Hou H et al. Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. *Brain Res* 2008 June 12;1214:177-87.

(276) Lee JW, Lee YK, Ban JO et al. Green tea (-)-epigallocatechin-3-gallate inhibits beta-amyloid-induced cognitive dysfunction through modification of secretase activity via inhibition of ERK and NF-kappaB pathways in mice. *J Nutr* 2009 October;139(10):1987-93.

(277) Choi YT, Jung CH, Lee SR et al. The green tea polyphenol (-)-epigallocatechin gallate attenuates beta-amyloid-induced neurotoxicity in cultured hippocampal neurons. *Life Sci* 2001 December 21;70(5):603-14.

(278) Lin CL, Chen TF, Chiu MJ, Way TD, Lin JK. Epigallocatechin gallate (EGCG) suppresses beta-amyloid-induced neurotoxicity through inhibiting c-Abl/FE65 nuclear translocation and GSK3 beta activation. *Neurobiol Aging* 2009 January;30(1):81-92.

(279) Kim CY, Lee C, Park GH, Jang JH. Neuroprotective effect of epigallocatechin-3-gallate against beta-amyloid-induced oxidative and nitrosative cell death via augmentation of antioxidant defense capacity. *Arch Pharm Res* 2009 June;32(6):869-81.

(280) He Y, Cui J, Lee JC et al. Prolonged exposure of cortical neurons to oligomeric amyloid-beta impairs NMDA receptor function via NADPH oxidase-mediated ROS production: protective effect of green tea (-)-epigallocatechin-3-gallate. *ASN Neuro* 2011;3(1):e00050.

(281) Dragicevic N, Smith A, Lin X et al. Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. *J Alzheimers Dis* 2011;26(3):507-21.

(282) Lee HP, Zhu X, Casadesus G et al. Antioxidant approaches for the treatment of Alzheimer's disease. *Expert Rev Neurother* 2010 July;10(7):1201-8.

(283) Dumont M, Beal MF. Neuroprotective strategies involving ROS in Alzheimer disease. *Free Radic Biol Med* 2011 September 1;51(5):1014-26.

(284) Yang CS, Lambert JD, Hou Z, Ju J, Lu G, Hao X. Molecular targets for the cancer preventive activity of tea polyphenols. *Mol Carcinog* 2006 June;45(6):431-5.

(285) Boehm K, Borrelli F, Ernst E et al. Green tea (Camellia sinensis) for the prevention of cancer. *Cochrane Database Syst Rev* 2009;(3):CD005004.

(286) Ogunleye AA, Xue F, Michels KB. Green tea consumption and breast cancer risk or recurrence: a meta-analysis. *Breast Cancer Res Treat* 2010 January;119(2):477-84.

(287) Shen CL, Yeh JK, Cao JJ, Chyu MC, Wang JS. Green tea and bone health: Evidence from laboratory studies. *Pharmacol Res* 2011 August;64(2):155-61.

(288) Rains TM, Agarwal S, Maki KC. Antiobesity effects of green tea catechins: a mechanistic review. *J Nutr Biochem* 2011 January;22(1):1-7.

(289) Ichinose T, Nomura S, Someya Y, Akimoto S, Tachiyashiki K, Imaizumi K. Effect of endurance training supplemented with green tea extract on substrate metabolism during exercise in humans. *Scand J Med Sci Sports* 2011 August;21(4):598-605.

(290) Wang H, Wen Y, Du Y et al. Effects of catechin enriched green tea on body composition. *Obesity (Silver Spring)* 2010 April;18(4):773-9.

(291) Maki KC, Reeves MS, Farmer M et al. Green tea catechin consumption enhances exercise-induced abdominal fat loss in overweight and obese adults. *J Nutr* 2009 February;139(2):264-70.

(292) Nagao T, Hase T, Tokimitsu I. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity (Silver Spring)* 2007 June;15(6):1473-83.

(293) Rodriguez C, Mayo JC, Sainz RM et al. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 2004 January;36(1):1-9.

(294) Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. *J Biomed Sci* 2000 November;7(6):444-58.

(295) Pioli C, Caroleo MC, Nistico G, Doria G. Melatonin increases antigen presentation and amplifies specific and non specific signals for T-cell proliferation. *Int J Immunopharmacol* 1993 May;15(4):463-8.

(296) Srinivasan V, Maestroni GJ, Cardinali DP, Esquifino AI, Perumal SR, Miller SC. Melatonin, immune function and aging. *Immun Ageing* 2005;2:17.

(297) Miller SC, Pandi-Perumal SR, Esquifino AI, Cardinali DP, Maestroni GJ. The role of melatonin in immuno-enhancement: potential application in cancer. *Int J Exp Pathol* 2006 April;87(2):81-7.

(298) Lissoni P. Is there a role for melatonin in supportive care? *Support Care Cancer* 2002 March;10(2):110-6.

(299) Lissoni P. Biochemotherapy with standard chemotherapies plus the pineal hormone melatonin in the treatment of advanced solid neoplasms. *Pathol Biol (Paris)* 2007 April;55(3-4):201-4.

(300) Lissoni P, Brivio F, Fumagalli L et al. Neuroimmunomodulation in medical oncology: application of psychoneuroimmunology with subcutaneous low-dose IL-2 and the pineal hormone melatonin in patients with untreatable metastatic solid tumors. *Anticancer Res* 2008 March;28(2B):1377-81.

(301) Srinivasan V, Maestroni GJ, Cardinali DP, Esquifino AI, Perumal SR, Miller SC. Melatonin, immune function and aging. *Immun Ageing* 2005;2:17.

(302) Cervantes M, Morali G, Letechipia-Vallejo G. Melatonin and ischemia-reperfusion injury of the brain. *J Pineal Res* 2008 August;45(1):1-7.

(303) Kurcer Z, Parlakpinar H, Vardi N et al. Protective effects of chronic melatonin treatment against renal ischemia/reperfusion injury in streptozotocin-induced diabetic rats. *Exp Clin Endocrinol Diabetes* 2007 June;115(6):365-71.

(304) Kim SH, Lee SM. Cytoprotective effects of melatonin against necrosis and apoptosis induced by ischemia/reperfusion injury in rat liver. *J Pineal Res* 2008 March;44(2):165-71.

(305) Korkmaz A, Oyar EO, Kardes O, Omeroglu S. Effects of melatonin on ischemic spinal cord injury caused by aortic cross clamping in rabbits. *Curr Neurovasc Res* 2008 February;5(1):46-51.

(306) Reiter RJ, Cabrera J, Sainz RM, Mayo JC, Manchester LC, Tan DX. Melatonin as a pharmacological agent against neuronal loss in experimental models of Huntington's disease, Alzheimer's disease and parkinsonism. *Ann N Y Acad Sci* 1999;890:471-85.

(307) Srinivasan V, Pandi-Perumal SR, Maestroni GJ, Esquifino AI, Hardeland R, Cardinali DP. Role of melatonin in neurodegenerative diseases. *Neurotox Res* 2005;7(4):293-318.

(308) Sudnikovich EJ, Maksimchik YZ, Zabrodskaya SV et al. Melatonin attenuates metabolic disorders due to streptozotocin-induced diabetes in rats. *Eur J Pharmacol* 2007 August 27;569(3):180-7.

(309) Moinova HR, Mulcahy RT. Up-regulation of the human gamma-glutamylcysteine synthetase regulatory subunit gene involves binding of Nrf-2 to an electrophile responsive element. *Biochem Biophys Res Commun* 1999 August 11;261(3):661-8.

(310) Wild AC, Moinova HR, Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *J Biol Chem* 1999 November 19;274(47):33627-36.

(311) Reiter RJ, cuna-Castroviejo D, Tan DX, Burkhardt S. Free radical-mediated molecular damage. Mechanisms for the protective actions of melatonin in the central nervous system. *Ann N Y Acad Sci* 2001 June;939:200-15.

(312) Urata Y, Honma S, Goto S et al. Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Radic Biol Med* 1999 October;27(7-8):838-47.

(313) Franco R, Schoneveld OJ, Pappa A, Panayiotidis MI. The central role of glutathione in the pathophysiology of human diseases. *Arch Physiol Biochem* 2007 October;113(4-5):234-58.

(314) Biswas SK, Rahman I. Environmental toxicity, redox signaling and lung inflammation: The role of glutathione. *Mol Aspects Med* 2008 August 8.

(315) Fernandes AP, Holmgren A. Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. *Antioxid Redox Signal* 2004 February;6(1):63-74.

(316) Stadtman TC. Selenium biochemistry. Mammalian selenoenzymes. *Ann N Y Acad Sci* 2000;899:399-402.

(317) Rahman I, Mulier B, Gilmour PS et al. Oxidant-mediated lung epithelial cell tolerance: the role of intracellular glutathione and nuclear factor-kappaB. *Biochem Pharmacol* 2001 September 15;62(6):787-94.

(318) Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annu Rev Pharmacol Toxicol* 2005;45:51-88.

(319) Pool-Zobel B, Veeriah S, Bohmer FD. Modulation of xenobiotic metabolising enzymes by anticarcinogens -- focus on glutathione S-transferases and their role as targets of dietary chemoprevention in colorectal carcinogenesis. *Mutat Res* 2005 December 11;591(1-2):74-92.

(320) De Rosa SC, Zaretsky MD, Dubs JG et al. N-acetylcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest* 2000 October;30(10):915-29.

(321) Atkuri KR, Mantovani JJ, Herzenberg LA, Herzenberg LA. N-Acetylcysteine--a safe antidote for cysteine/glutathione deficiency. *Curr Opin Pharmacol* 2007 August;7(4):355-9.

(322) Dodd S, Dean O, Copolov DL, Malhi GS, Berk M. N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. *Expert Opin Biol Ther* 2008 December;8(12):1955-62.

(323) Ziment I. Acetylcysteine: a drug with an interesting past and a fascinating future. *Respiration* 1986;50 Suppl 1:26-30.

(324) Heard KJ. Acetylcysteine for acetaminophen poisoning. *N Engl J Med* 2008 July 17;359(3):285-92.

(325) Sadowska AM, Verbraecken J, Darquennes K, De Backer WA. Role of N-acetylcysteine in the management of COPD. *Int J Chron Obstruct Pulmon Dis* 2006;1(4):425-34.

(326) Dekhuijzen PN, van Beurden WJ. The role for N-acetylcysteine in the management of COPD. *Int J Chron Obstruct Pulmon Dis* 2006;1(2):99-106.

(327) Demedts M, Behr J, Buhl R et al. High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med* 2005 November 24;353(21):2229-42.

(328) Ghanei M, Shohrati M, Jafari M, Ghaderi S, Alaeddini F, Aslani J. N-acetylcysteine improves the clinical conditions of mustard gas-exposed patients with normal pulmonary function test. *Basic Clin Pharmacol Toxicol* 2008 November;103(5):428-32.

(329) Delgado JL, Landeras J, Carbonell LF et al. Effect of N-acetylcysteine on vascular endothelium function in aorta from oophorectomized rats. *Gen Pharmacol* 1999 January;32(1):23-7.

(330) Martina V, Masha A, Gigliardi VR et al. Long-term N-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes. *Diabetes Care* 2008 May;31(5):940-4.

(331) Sahin G, Yalcin AU, Akcar N. Effect of N-acetylcysteine on endothelial dysfunction in dialysis patients. *Blood Purif* 2007;25(4):309-15.

(332) Pieper GM, Siebeneich W. Oral administration of the antioxidant, N-acetylcysteine, abrogates diabetes-induced endothelial dysfunction. *J Cardiovasc Pharmacol* 1998 July;32(1):101-5.

(333) Xia Z, Kuo KH, Nagareddy PR et al. N-acetylcysteine attenuates PKCbeta2 overexpression and myocardial hypertrophy in streptozotocin-induced diabetic rats. *Cardiovasc Res* 2007 March 1;73(4):770-82.

(334) Ozkilic AC, Cengiz M, Ozaydin A, Cobanoglu A, Kanigur G. The role of N-acetylcysteine treatment on anti-oxidative status in patients with type II diabetes mellitus. *J Basic Clin Physiol Pharmacol* 2006;17(4):245-54.

(335) Sagara M, Satoh J, Wada R et al. Inhibition of development of peripheral neuropathy in streptozotocin-induced diabetic rats with N-acetylcysteine. *Diabetologia* 1996 March;39(3):263-9.

(336) Arakawa M, Ito Y. N-acetylcysteine and neurodegenerative diseases: Basic and clinical pharmacology. *Cerebellum* 2007 January 19;1-7.

(337) Berk M, Ng F, Dean O, Dodd S, Bush AI. Glutathione: a novel treatment target in psychiatry. *Trends Pharmacol Sci* 2008 July;29(7):346-51.

(338) Akerlund B, Jarstrand C, Lindeke B, Sonnerborg A, Akerblad AC, Rasool O. Effect of N-acetylcysteine(NAC) treatment on HIV-1 infection: a double-blind placebo-controlled trial. *Eur J Clin Pharmacol* 1996;50(6):457-61.

(339) De FS, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. *Eur Respir J* 1997 July;10(7):1535-41.

(340) Ungheri D, Pisani C, Sanson G et al. Protective effect of n-acetylcysteine in a model of influenza infection in mice. *Int J Immunopathol Pharmacol* 2000 September;13(3):123-8.

(341) Ghezzi P, Ungheri D. Synergistic combination of N-acetylcysteine and ribavirin to protect from lethal influenza viral infection in a mouse model. *Int J Immunopathol Pharmacol* 2004 January;17(1):99-102.

(342) Garozzo A, Tempera G, Ungheri D, Timpanaro R, Castro A. N-acetylcysteine synergizes with oseltamivir in protecting mice from lethal influenza infection. *Int J Immunopathol Pharmacol* 2007 April;20(2):349-54.

(343) Knobil K, Choi AM, Weigand GW, Jacoby DB. Role of oxidants in influenza virus-induced gene expression. *Am J Physiol* 1998 January;274(1 Pt 1):L134-L142.

(344) Moreira PI, Harris PL, Zhu X et al. Lipoic acid and N-acetyl cysteine decrease mitochondrial-related oxidative stress in Alzheimer disease patient fibroblasts. *J Alzheimers Dis* 2007 September;12(2):195-206.

(345) Baker DH. Comparative species utilization and toxicity of sulfur amino acids. *J Nutr* 2006 June;136(6 Suppl):1670S-5S.

(346) Murakami S, Kurihara S, Titchenal CA, Ohtani M. Suppression of exercise-induced neutrophilia and lymphopenia in athletes by cystine/theanine intake: a randomized, double-blind, placebo-controlled trial. *J Int Soc Sports Nutr* 2010;7(1):23.

(347) Williams RJ. *Nutrition against disease.* Bantam; 1973.

(348) McCarty MF. Point of view--a role for "nutritional insurance" supplementation in preventive medicine. *Med Hypotheses* 1981 February;7(2):171-85.

(349) Stadtman TC. Selenium biochemistry. Mammalian selenoenzymes. *Ann N Y Acad Sci* 2000;899:399-402.

(350) Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, Guallar E. Selenium and coronary heart disease: a meta-analysis. *Am J Clin Nutr* 2006 October;84(4):762-73.

(351) Stranges S, Marshall JR, Trevisan M et al. Effects of selenium supplementation on cardiovascular disease incidence and mortality: secondary analyses in a randomized clinical trial. *Am J Epidemiol* 2006 April 15;163(8):694-9.

(352) Alehagen U, Johansson P, Bjornstedt M, Rosen A, Dahlstrom U. Cardiovascular mortality and N-terminal-proBNP reduced after combined selenium and coenzyme Q10 supplementation: A 5-year prospective randomized double-blind placebo-controlled trial among elderly Swedish citizens. *Int J Cardiol* 2012 May 22.

(353) Griffin AC. Role of selenium in the chemoprevention of cancer. *Adv Cancer Res* 1979;29:419-42.

(354) Ip C, Thompson HJ, Zhu Z, Ganther HE. In vitro and in vivo studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. *Cancer Res* 2000 June 1;60(11):2882-6.

(355) Ganther HE. Selenium metabolism and mechanisms of cancer prevention. *Adv Exp Med Biol* 2001;492:119-30.

(356) Sinha R, el-Bayoumy K. Apoptosis is a critical cellular event in cancer chemoprevention and chemotherapy by selenium compounds. *Curr Cancer Drug Targets* 2004 February;4(1):13-28.

(357) Smith ML, Lancia JK, Mercer TI, Ip C. Selenium compounds regulate p53 by common and distinctive mechanisms. *Anticancer Res* 2004 May;24(3a):1401-8.

(358) Waters DJ, Shen S, Cooley DM et al. Effects of dietary selenium supplementation on DNA damage and apoptosis in canine prostate. *J Natl Cancer Inst* 2003 February 5;95(3):237-41.

(359) Clark LC, Combs GF, Jr., Turnbull BW et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* 1996 December 25;276(24):1957-63.

(360) Duffield-Lillico AJ, Reid ME, Turnbull BW et al. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. *Cancer Epidemiol Biomarkers Prev* 2002 July;11(7):630-9.

(361) Duffield-Lillico AJ, Dalkin BL, Reid ME et al. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete

treatment period of the Nutritional Prevention of Cancer Trial. *BJU Int* 2003 May;91(7):608-12.

(362) Lener M, Jaworska K, Muszynska M et al. Selenium as marker for cancer risk and prevention. *Pol Przegl Chir* 2012 October 1;84(9):470-5.

(363) Reid ME, Duffield-Lillico AJ, Slate E et al. The nutritional prevention of cancer: 400 mcg per day selenium treatment. *Nutr Cancer* 2008 March;60(2):155-63.

(364) Lippman SM, Goodman PJ, Klein EA et al. Designing the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *J Natl Cancer Inst* 2005 January 19;97(2):94-102.

(365) Lee EH, Myung SK, Jeon YJ et al. Effects of selenium supplements on cancer prevention: meta-analysis of randomized controlled trials. *Nutr Cancer* 2011 November;63(8):1185-95.

(366) Mello Filho AC, Meneghini R. In vivo formation of single-strand breaks in DNA by hydrogen peroxide is mediated by the Haber-Weiss reaction. *Biochim Biophys Acta* 1984 February 24;781(1-2):56-63.

(367) Powell SR. The antioxidant properties of zinc. *J Nutr* 2000 May;130(5S Suppl):1447S-54S.

(368) Sullivan VK, Burnett FR, Cousins RJ. Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. *J Nutr* 1998 April;128(4):707-13.

(369) Cao J, Cousins RJ. Metallothionein mRNA in monocytes and peripheral blood mononuclear cells and in cells from dried blood spots increases after zinc supplementation of men. *J Nutr* 2000 September;130(9):2180-7.

(370) Aydemir TB, Blanchard RK, Cousins RJ. Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations. *Proc Natl Acad Sci U S A* 2006 February 7;103(6):1699-704.

(371) Liu Y, Liu J, Habeebu SM, Waalkes MP, Klaassen CD. Metallothionein-I/II null mice are sensitive to chronic oral cadmium-induced nephrotoxicity. *Toxicol Sci* 2000 September;57(1):167-76.

(372) Klaassen CD, Liu J, Diwan BA. Metallothionein protection of cadmium toxicity. *Toxicol Appl Pharmacol* 2009 August 1;238(3):215-20.

(373) Jarup L, Akesson A. Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol* 2009 August 1;238(3):201-8.

(374) Nawrot TS, Staessen JA, Roels HA et al. Cadmium exposure in the population: from health risks to strategies of prevention. *Biometals* 2010 October;23(5):769-82.

(375) Gallagher CM, Chen JJ, Kovach JS. Environmental cadmium and breast cancer risk. *Aging (Albany NY)* 2010 November;2(11):804-14.

(376) Adams SV, Passarelli MN, Newcomb PA. Cadmium exposure and cancer mortality in the Third National Health and Nutrition Examination Survey cohort. *Occup Environ Med* 2012 February;69(2):153-6.

(377) Cuypers A, Plusquin M, Remans T et al. Cadmium stress: an oxidative challenge. *Biometals* 2010 October;23(5):927-40.

(378) Bulat ZP, Djukic-Cosic D, Malicevic Z, Bulat P, Matovic V. Zinc or magnesium supplementation modulates cd intoxication in blood, kidney, spleen, and bone of rabbits. *Biol Trace Elem Res* 2008 August;124(2):110-7.

(379) Brzoska MM, Roszczenko A, Galazyn-Sidorczuk M, Majewska K. Zinc supplementation can protect from enhanced risk of femoral neck fracture in male rats chronically exposed to cadmium. *Exp Toxicol Pathol* 2011 July;63(5):491-8.

(380) Rogalska J, Pilat-Marcinkiewicz B, Brzoska MM. Protective effect of zinc against cadmium hepatotoxicity depends on this bioelement intake and level of cadmium exposure: a study in a rat model. *Chem Biol Interact* 2011 September 30;193(3):191-203.

(381) Paniagua-Castro N, Escalona-Cardoso G, Hernandez-Navarro D, Perez-Pasten R, Chamorro-Cevallos G. Spirulina (Arthrospira) protects against cadmium-induced teratogenic damage in mice. *J Med Food* 2011 April;14(4):398-404.

(382) Ryu DY, Lee SJ, Park DW, Choi BS, Klaassen CD, Park JD. Dietary iron regulates intestinal cadmium absorption through iron transporters in rats. *Toxicol Lett* 2004 August 30;152(1):19-25.

(383) Vesey DA. Transport pathways for cadmium in the intestine and kidney proximal tubule: focus on the interaction with essential metals. *Toxicol Lett* 2010 September 15;198(1):13-9.

(384) Min KS, Ueda H, Kihara T, Tanaka K. Increased hepatic accumulation of ingested Cd is associated with upregulation of several intestinal transporters in mice fed diets deficient in essential metals. *Toxicol Sci* 2008 November;106(1):284-9.

(385) Reeves PG, Chaney RL. Marginal nutritional status of zinc, iron, and calcium increases cadmium retention in the duodenum and other organs of rats fed rice-based diets. *Environ Res* 2004 November;96(3):311-22.

(386) Gallagher CM, Chen JJ, Kovach JS. The relationship between body iron stores and blood and urine cadmium concentrations in US never-smoking, non-pregnant women aged 20-49 years. *Environ Res* 2011 July;111(5):702-7.

(387) Min KS, Ueda H, Tanaka K. Involvement of intestinal calcium transporter 1 and metallothionein in cadmium accumulation in the liver and kidney of mice fed a low-calcium diet. *Toxicol Lett* 2008 January 4;176(1):85-92.

(388) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol* 2001 October;119(10):1417-36.

(389) Clemons TE, Kurinij N, Sperduto RD. Associations of mortality with ocular disorders and an intervention of high-dose antioxidants and zinc in the Age-Related Eye Disease Study: AREDS Report No. 13. *Arch Ophthalmol* 2004 May;122(5):716-26.

(390) Prasad AS, Beck FW, Bao B et al. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *Am J Clin Nutr* 2007 March;85(3):837-44.

(391) Bao B, Prasad AS, Beck FW et al. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. *Am J Clin Nutr* 2010 June;91(6):1634-41.

(392) Foster M, Petocz P, Samman S. Effects of zinc on plasma lipoprotein cholesterol concentrations in humans: a meta-analysis of randomised controlled trials. *Atherosclerosis* 2010 June;210(2):344-52.

(393) Prasad AS, Brewer GJ, Schoomaker EB, Rabbani P. Hypocupremia induced by zinc therapy in adults. *JAMA* 1978 November 10;240(20):2166-8.

(394) Meagher EA, Barry OP, Lawson JA, Rokach J, FitzGerald GA. Effects of vitamin E on lipid peroxidation in healthy persons. *JAMA* 2001 March 7;285(9):1178-82.

(395) Steinberg D. Clinical trials of antioxidants in atherosclerosis: are we doing the right thing? *Lancet* 1995 July 1;346(8966):36-8.

(396) Shekelle PG, Morton SC, Jungvig LK et al. Effect of supplemental vitamin E for the prevention and treatment of cardiovascular disease. *J Gen Intern Med* 2004 April;19(4):380-9.

(397) Sesso HD, Buring JE, Christen WG et al. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA* 2008 November 12;300(18):2123-33.

(398) Miller ER, III, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* 2005 January 4;142(1):37-46.

(399) Devaraj S, Jialal I. Failure of vitamin E in clinical trials: is gamma-tocopherol the answer? *Nutr Rev* 2005 August;63(8):290-3.

(400) Christen S, Woodall AA, Shigenaga MK, Southwell-Keely PT, Duncan MW, Ames BN. gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications. *Proc Natl Acad Sci U S A* 1997 April 1;94(7):3217-22.

(401) Jiang Q, Christen S, Shigenaga MK, Ames BN. gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. *Am J Clin Nutr* 2001 December;74(6):714-22.

(402) Jiang Q, Lykkesfeldt J, Shigenaga MK, Shigeno ET, Christen S, Ames BN. Gamma-tocopherol supplementation inhibits protein nitration and ascorbate oxidation in rats with inflammation. *Free Radic Biol Med* 2002 December 1;33(11):1534-42.

(403) Jiang Q, Ames BN. Gamma-tocopherol, but not alpha-tocopherol, decreases proinflammatory eicosanoids and inflammation damage in rats. *FASEB J* 2003 May;17(8):816-22.

(404) Wolf G. How an increased intake of alpha-tocopherol can suppress the bioavailability of gamma-tocopherol. *Nutr Rev* 2006 June;64(6):295-9.

(405) Jiang Q, Christen S, Shigenaga MK, Ames BN. gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. *Am J Clin Nutr* 2001 December;74(6):714-22.

(406) Klein EA, Thompson IM, Jr., Tangen CM et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2011 October 12;306(14):1549-56.

(407) Barve A, Khor TO, Nair S et al. Gamma-tocopherol-enriched mixed tocopherol diet inhibits prostate carcinogenesis in TRAMP mice. *Int J Cancer* 2009 April 1;124(7):1693-9.

(408) Takahashi S, Takeshita K, Seeni A et al. Suppression of prostate cancer in a transgenic rat model via gamma-tocopherol activation of caspase signaling. *Prostate* 2009 May 1;69(6):644-51.

(409) Galli F, Stabile AM, Betti M et al. The effect of alpha- and gamma-tocopherol and their carboxyethyl hydroxychroman metabolites on prostate cancer cell proliferation. *Arch Biochem Biophys* 2004 March 1;423(1):97-102.

(410) Jiang Q, Wong J, Fyrst H, Saba JD, Ames BN. gamma-Tocopherol or combinations of vitamin E forms induce cell death in human prostate cancer cells by interrupting sphingolipid synthesis. *Proc Natl Acad Sci U S A* 2004 December 21;101(51):17825-30.

(411) Jiang Q, Wong J, Ames BN. Gamma-tocopherol induces apoptosis in androgen-responsive LNCaP prostate cancer cells via caspase-dependent and independent mechanisms. *Ann N Y Acad Sci* 2004 December;1031:399-400.

(412) Campbell SE, Musich PR, Whaley SG et al. Gamma tocopherol upregulates the expression of 15-S-HETE and induces growth arrest through a PPAR gamma-dependent mechanism in PC-3 human prostate cancer cells. *Nutr Cancer* 2009;61(5):649-62.

(413) Helzlsouer KJ, Huang HY, Alberg AJ et al. Association between alpha-tocopherol, gamma-tocopherol, selenium, and subsequent prostate cancer. *J Natl Cancer Inst* 2000 December 20;92(24):2018-23.

(414) Giovannucci E. Gamma-tocopherol: a new player in prostate cancer prevention? *J Natl Cancer Inst* 2000 December 20;92(24):1966-7.

(415) Weinstein SJ, Wright ME, Pietinen P et al. Serum alpha-tocopherol and gamma-tocopherol in relation to prostate cancer risk in a prospective study. *J Natl Cancer Inst* 2005 March 2;97(5):396-9.

(416) Wright ME, Weinstein SJ, Lawson KA et al. Supplemental and dietary vitamin E intakes and risk of prostate cancer in a large prospective study. *Cancer Epidemiol Biomarkers Prev* 2007 June;16(6):1128-35.

(417) Devaraj S, Leonard S, Traber MG, Jialal I. Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radic Biol Med* 2008 March 15;44(6):1203-8.

(418) Singh I, Turner AH, Sinclair AJ, Li D, Hawley JA. Effects of gamma-tocopherol supplementation on thrombotic risk factors. *Asia Pac J Clin Nutr* 2007;16(3):422-8.

(419) Wagner JG, Jiang Q, Harkema JR et al. Ozone enhancement of lower airway allergic inflammation is prevented by gamma-tocopherol. *Free Radic Biol Med* 2007 October 15;43(8):1176-88.

(420) Wagner JG, Jiang Q, Harkema JR et al. Gamma-tocopherol prevents airway eosinophilia and mucous cell hyperplasia in experimentally induced allergic rhinitis and asthma. *Clin Exp Allergy* 2008 March;38(3):501-11.

(421) Lavine JE. Vitamin E treatment of nonalcoholic steatohepatitis in children: a pilot study. *J Pediatr* 2000 June;136(6):734-8.

(422) Madan K, Batra Y, Gupta DS et al. Vitamin E-based therapy is effective in ameliorating transaminasemia in nonalcoholic fatty liver disease. *Indian J Gastroenterol* 2005 November;24(6):251-5.

(423) Sanyal AJ, Chalasani N, Kowdley KV et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010 May 6;362(18):1675-85.

(424) Heller R, Unbehaun A, Schellenberg B, Mayer B, Werner-felmayer G, Werner ER. L-ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin. *J Biol Chem* 2001 January 5;276(1):40-7.

(425) Heller R, Munscher-Paulig F, Grabner R, Till U. L-Ascorbic acid potentiates nitric oxide synthesis in endothelial cells. *J Biol Chem* 1999 March 19;274(12):8254-60.

(426) Ek A, Strom K, Cotgreave IA. The uptake of ascorbic acid into human umbilical vein endothelial cells and its effect on oxidant insult. *Biochem Pharmacol* 1995 October 26;50(9):1339-46.

(427) Levine M, Conry-Cantilena C, Wang Y et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci U S A* 1996 April 16;93(8):3704-9.

(428) Douglas RM, Hemila H, Chalker E, Treacy B. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst Rev* 2007;(3):CD000980.

(429) Chen Q, Espey MG, Sun AY et al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. *Proc Natl Acad Sci U S A* 2007 May 22;104(21):8749-54.

(430) Chen Q, Espey MG, Krishna MC et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc Natl Acad Sci U S A* 2005 September 20;102(38):13604-9.

(431) Padayatty SJ, Riordan HD, Hewitt SM, Katz A, Hoffer LJ, Levine M. Intravenously administered vitamin C as cancer therapy: three cases. *CMAJ* 2006 March 28;174(7):937-42.

(432) McCarty MF, Barroso-Aranda J, Contreras F. A two-phase strategy for treatment of oxidant-dependent cancers. *Med Hypotheses* 2007;69(3):489-96.

(433) Ranzato E, Biffo S, Burlando B. Selective ascorbate toxicity in malignant mesothelioma: a redox Trojan mechanism. *Am J Respir Cell Mol Biol* 2011 January;44(1):108-17.

(434) Verrax J, Calderon PB. Pharmacologic concentrations of ascorbate are achieved by parenteral administration and exhibit antitumoral effects. *Free Radic Biol Med* 2009 July 1;47(1):32-40.

(435) Espey MG, Chen P, Chalmers B et al. Pharmacologic ascorbate synergizes with gemcitabine in preclinical models of pancreatic cancer. *Free Radic Biol Med* 2011 June 1;50(11):1610-9.

(436) Rivas CI, Zuniga FA, Salas-Burgos A, Mardones L, Ormazabal V, Vera JC. Vitamin C transporters. *J Physiol Biochem* 2008 December;64(4):357-75.

(437) Huang J, Agus DB, Winfree CJ et al. Dehydroascorbic acid, a blood-brain barrier transportable form of vitamin C, mediates potent cerebroprotection in experimental stroke. *Proc Natl Acad Sci U S A* 2001 September 25;98(20):11720-4.

(438) De T, V, Brizzi S, Saviozzi M et al. Protective role of dehydroascorbate in rat liver ischemia-reperfusion injury. *J Surg Res* 2005 February;123(2):215-21.

(439) Mack WJ, Mocco J, Ducruet AF et al. A cerebroprotective dose of intravenous citrate/sorbitol-stabilized dehydroascorbic acid is correlated with increased cerebral ascorbic acid and inhibited lipid peroxidation after murine reperfused stroke. *Neurosurgery* 2006 August;59(2):383-8.

(440) Andrae U, Singh J, Ziegler-Skylakakis K. Pyruvate and related alpha-ketoacids protect mammalian cells in culture against hydrogen peroxide-induced cytotoxicity. *Toxicol Lett* 1985 November;28(2-3):93-8.

(441) Fink MP. Reactive oxygen species as mediators of organ dysfunction caused by sepsis, acute respiratory distress syndrome, or hemorrhagic shock: potential benefits of resuscitation with Ringer's ethyl pyruvate solution. *Curr Opin Clin Nutr Metab Care* 2002 March;5(2):167-74.

(442) Fink MP. Ethyl pyruvate. *Curr Opin Anaesthesiol* 2008 April;21(2):160-7.

(443) Melhus H, Michaelsson K, Kindmark A et al. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. *Ann Intern Med* 1998 November 15;129(10):770-8.

(444) Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. *JAMA* 2002 January 2;287(1):47-54.

(445) Kochevar IE. Singlet oxygen signaling: from intimate to global. *Sci STKE* 2004 February 24;2004(221):e7.

(446) Valencia A, Kochevar IE. Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes. *J Invest Dermatol* 2008 January;128(1):214-22.

(447) Seddon JM, Ajani UA, Sperduto RD et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA* 1994 November 9;272(18):1413-20.

(448) Stringham JM, Hammond BR, Jr. Dietary lutein and zeaxanthin: possible effects on visual function. *Nutr Rev* 2005 February;63(2):59-64.

(449) Giovannucci E. A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Exp Biol Med (Maywood)* 2002 November;227(10):852-9.

(450) Barber NJ, Zhang X, Zhu G et al. Lycopene inhibits DNA synthesis in primary prostate epithelial cells in vitro and its administration is associated with a reduced prostate-specific antigen velocity in a phase II clinical study. *Prostate Cancer Prostatic Dis* 2006;9(4):407-13.

(451) Ellinger S, Ellinger J, Stehle P. Tomatoes, tomato products and lycopene in the prevention and treatment of prostate cancer: do we have the evidence from intervention studies? *Curr Opin Clin Nutr Metab Care* 2006 November;9(6):722-7.

(452) Schwarz S, Obermuller-Jevic UC, Hellmis E, Koch W, Jacobi G, Biesalski HK. Lycopene inhibits disease progression in patients with benign prostate hyperplasia. *J Nutr* 2008 January;138(1):49-53.

(453) Liu C, Lian F, Smith DE, Russell RM, Wang XD. Lycopene supplementation inhibits lung squamous metaplasia and induces apoptosis via up-regulating insulin-like growth factor-binding protein 3 in cigarette smoke-exposed ferrets. *Cancer Res* 2003 June 15;63(12):3138-44.

(454) Kanagaraj P, Vijayababu MR, Ravisankar B, Anbalagan J, Aruldhas MM, Arunakaran J. Effect of lycopene on insulin-like growth factor-I, IGF binding protein-3 and IGF type-I receptor in prostate cancer cells. *J Cancer Res Clin Oncol* 2007 June;133(6):351-9.

(455) Graydon R, Gilchrist SE, Young IS, Obermuller-Jevic U, Hasselwander O, Woodside JV. Effect of lycopene supplementation on insulin-like growth factor-1 and insulin-like growth factor binding protein-3: a double-blind, placebo-controlled trial. *Eur J Clin Nutr* 2007 October;61(10):1196-200.

(456) Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Mol Aspects Med* 2005 December;26(6):459-516.

(457) Wane D, Lengacher CA. Integrative review of lycopene and breast cancer. *Oncol Nurs Forum* 2006 January;33(1):127-37.

(458) Seren S, Lieberman R, Bayraktar UD et al. Lycopene in cancer prevention and treatment. *Am J Ther* 2008 January;15(1):66-81.

(459) Young CY, Yuan HQ, He ML, Zhang JY. Carotenoids and prostate cancer risk. *Mini Rev Med Chem* 2008 May;8(5):529-37.

(460) Gallicchio L, Boyd K, Matanoski G et al. Carotenoids and the risk of developing lung cancer: a systematic review. *Am J Clin Nutr* 2008 August;88(2):372-83.

(461) Albanes D, Heinonen OP, Taylor PR et al. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst* 1996 November 6;88(21):1560-70.

(462) Liu C, Russell RM, Wang XD. Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. *J Nutr* 2003 January;133(1):173-9.

(463) Liu C, Russell RM, Wang XD. Alpha-tocopherol and ascorbic acid decrease the production of beta-apo-carotenals and increase the formation of retinoids from beta-carotene in the lung tissues of cigarette smoke-exposed ferrets in vitro. *J Nutr* 2004 February;134(2):426-30.

(464) Kim Y, Chongviriyaphan N, Liu C, Russell RM, Wang XD. Combined antioxidant (beta-carotene, alpha-tocopherol and ascorbic acid) supplementation increases the levels of lung retinoic acid and inhibits the activation of mitogen-activated protein kinase in the ferret lung cancer model. *Carcinogenesis* 2006 July;27(7):1410-9.

(465) Kikuchi A, Yamaya M, Suzuki S et al. Association of susceptibility to the development of lung adenocarcinoma with the heme oxygenase-1 gene promoter polymorphism. *Hum Genet* 2005 April;116(5):354-60.

(466) Yoshie Y, Ohshima H. Synergistic induction of DNA strand breakage by cigarette tar and nitric oxide. *Carcinogenesis* 1997 July;18(7):1359-63.

(467) Schroeter H, Heiss C, Balzer J et al. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc Natl Acad Sci U S A* 2006 January 24;103(4):1024-9.

(468) Fisher ND, Sorond FA, Hollenberg NK. Cocoa flavanols and brain perfusion. *J Cardiovasc Pharmacol* 2006;47 Suppl 2:S210-S214.

(469) Neukam K, Stahl W, Tronnier H, Sies H, Heinrich U. Consumption of flavanol-rich cocoa acutely increases microcirculation in human skin. *Eur J Nutr* 2007 February;46(1):53-6.

(470) Heinrich U, Neukam K, Tronnier H, Sies H, Stahl W. Long-term ingestion of high flavanol cocoa provides photoprotection against UV-induced erythema and improves skin condition in women. *J Nutr* 2006 June;136(6):1565-9.

(471) Grassi D, Necozione S, Lippi C et al. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension* 2005 August;46(2):398-405.

(472) Taubert D, Roesen R, Lehmann C, Jung N, Schomig E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. *JAMA* 2007 July 4;298(1):49-60.

(473) Grassi D, Desideri G, Necozione S et al. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr* 2008 September;138(9):1671-6.

(474) Bisson JF, Nejdi A, Rozan P, Hidalgo S, Lalonde R, Messaoudi M. Effects of long-term administration of a cocoa polyphenolic extract (Acticoa powder) on cognitive performances in aged rats. *Br J Nutr* 2008 July;100(1):94-101.

(475) Hollenberg NK, Martinez G, McCullough M et al. Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. *Hypertension* 1997 January;29(1 Pt 2):171-6.

(476) Bayard V, Chamorro F, Motta J, Hollenberg NK. Does flavanol intake influence mortality from nitric oxide-dependent processes? Ischemic heart disease, stroke, diabetes mellitus, and cancer in Panama. *Int J Med Sci* 2007;4(1):53-8.

(477) McCullough ML, Chevaux K, Jackson L et al. Hypertension, the Kuna, and the epidemiology of flavanols. *J Cardiovasc Pharmacol* 2006;47 Suppl 2:S103-S109.

(478) Loke WM, Hodgson JM, Proudfoot JM, McKinley AJ, Puddey IB, Croft KD. Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am J Clin Nutr* 2008 October;88(4):1018-25.

(479) Perez-Vizcaino F, Duarte J. Flavonols and cardiovascular disease. *Mol Aspects Med* 2010 September 15.

(480) Huxley RR, Neil HA. The relation between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. *Eur J Clin Nutr* 2003 August;57(8):904-8.

(481) Hollman PC, Geelen A, Kromhout D. Dietary flavonol intake may lower stroke risk in men and women. *J Nutr* 2010 March;140(3):600-4.

(482) Kuhlmann CR, Schaefer CA, Kosok C et al. Quercetin-induced induction of the NO/cGMP pathway depends on Ca2+-activated K+ channel-induced hyperpolarization-mediated Ca2+-entry into cultured human endothelial cells. *Planta Med* 2005 June;71(6):520-4.

(483) Nishida S, Satoh H. Possible Involvement of Ca Activated K Channels, SK Channel, in the Quercetin-Induced Vasodilatation. *Korean J Physiol Pharmacol* 2009 October;13(5):361-5.

(484) Steffen Y, Gruber C, Schewe T, Sies H. Mono-O-methylated flavanols and other flavonoids as inhibitors of endothelial NADPH oxidase. *Arch Biochem Biophys* 2008 January 15;469(2):209-19.

(485) Perez-Vizcaino F, Duarte J, Jimenez R, Santos-Buelga C, Osuna A. Antihypertensive effects of the flavonoid quercetin. *Pharmacol Rep* 2009 January;61(1):67-75.

(486) Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. Quercetin reduces blood pressure in hypertensive subjects. *J Nutr* 2007 November;137(11):2405-11.

(487) Juzwiak S, Wojcicki J, Mokrzycki K et al. Effect of quercetin on experimental hyperlipidemia and atherosclerosis in rabbits. *Pharmacol Rep* 2005 September;57(5):604-9.

(488) Loke WM, Proudfoot JM, Hodgson JM et al. Specific dietary polyphenols attenuate atherosclerosis in apolipoprotein E-knockout mice by alleviating inflammation and endothelial dysfunction. *Arterioscler Thromb Vasc Biol* 2010 April;30(4):749-57.

(489) Davis JM, Murphy EA, Carmichael MD, Davis B. Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *Am J Physiol Regul Integr Comp Physiol* 2009 April;296(4):R1071-R1077.

(490) Nieman DC, Williams AS, Shanely RA et al. Quercetin's influence on exercise performance and muscle mitochondrial biogenesis. *Med Sci Sports Exerc* 2010 February;42(2):338-45.

(491) Nisoli E, Clementi E, Paolucci C et al. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* 2003 February 7;299(5608):896-9.

(492) Nisoli E, Falcone S, Tonello C et al. Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. *Proc Natl Acad Sci U S A* 2004 November 23;101(47):16507-12.

(493) Davis JM, Carlstedt CJ, Chen S, Carmichael MD, Murphy EA. The dietary flavonoid quercetin increases VO(2max) and endurance capacity. *Int J Sport Nutr Exerc Metab* 2010 February;20(1):56-62.

(494) Nisoli E, Clementi E, Carruba MO, Moncada S. Defective mitochondrial biogenesis: a hallmark of the high cardiovascular risk in the metabolic syndrome? *Circ Res* 2007 March 30;100(6):795-806.

(495) Rivera L, Moron R, Sanchez M, Zarzuelo A, Galisteo M. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. *Obesity (Silver Spring)* 2008 September;16(9):2081-7.

(496) Okawara M, Katsuki H, Kurimoto E, Shibata H, Kume T, Akaike A. Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults. *Biochem Pharmacol* 2007 February 15;73(4):550-60.

(497) Suchankova G, Nelson LE, Gerhart-Hines Z et al. Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells. *Biochem Biophys Res Commun* 2009 January 23;378(4):836-41.

(498) McCarty MF, Barroso-Aranda J, Contreras F. Potential complementarity of high-flavanol cocoa powder and spirulina for health protection. *Med Hypotheses* 2010 February;74(2):370-3.

(499) Cooke JP. The pivotal role of nitric oxide for vascular health. *Can J Cardiol* 2004 August;20 Suppl B:7B-15B.

(500) Anderson TJ. Nitric oxide, atherosclerosis and the clinical relevance of endothelial dysfunction. *Heart Fail Rev* 2003 January;8(1):71-86.

(501) Ritchie RH, Irvine JC, Rosenkranz AC et al. Exploiting cGMP-based therapies for the prevention of left ventricular hypertrophy: NO* and beyond. *Pharmacol Ther* 2009 December;124(3):279-300.

(502) Austin SA, Santhanam AV, Katusic ZS. Endothelial Nitric Oxide Modulates Expression and Processing of Amyloid Precursor Protein. *Circ Res* 2010 December 2.

(503) Wimalawansa SJ. Nitric oxide and bone. *Ann N Y Acad Sci* 2010 March;1192:391-403.

(504) Whitsett J, Picklo MJ, Sr., Vasquez-Vivar J. 4-Hydroxy-2-nonenal increases superoxide anion radical in endothelial cells via stimulated GTP cyclohydrolase proteasomal degradation. *Arterioscler Thromb Vasc Biol* 2007 November;27(11):2340-7.

(505) Forbes SP, Druhan LJ, Guzman JE et al. Mechanism of 4-HNE mediated inhibition of hDDAH-1: implications in no regulation. *Biochemistry* 2008 February 12;47(6):1819-26.

(506) Luo Z, Teerlink T, Griendling K, Aslam S, Welch WJ, Wilcox CS. Angiotensin II and NADPH oxidase increase ADMA in vascular smooth muscle cells. *Hypertension* 2010 September;56(3):498-504.

(507) Laughlin MH, Pollock JS, Amann JF, Hollis ML, Woodman CR, Price EM. Training induces nonuniform increases in eNOS content along the coronary arterial tree. *J Appl Physiol* 2001 February;90(2):501-10.

(508) Green DJ, Maiorana A, O'Driscoll G, Taylor R. Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol* 2004 November 15;561(Pt 1):1-25.

(509) Gielen S, Sandri M, Erbs S, Adams V. Exercise-induced Modulation of Endothelial Nitric Oxide Production. *Curr Pharm Biotechnol* 2011 January 11.

(510) Waugh WH, Daeschner CW, III, Files BA, McConnell ME, Strandjord SE. Oral citrulline as arginine precursor may be beneficial in sickle cell disease: early phase two results. *J Natl Med Assoc* 2001 October;93(10):363-71.

(511) Schwedhelm E, Maas R, Freese R et al. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. *Br J Clin Pharmacol* 2008 January;65(1):51-9.

(512) Figueroa A, Trivino JA, Sanchez-Gonzalez MA, Vicil F. Oral L-citrulline supplementation attenuates blood pressure response to cold pressor test in young men. *Am J Hypertens* 2010 January;23(1):12-6.

(513) Ochiai M, Hayashi T, Morita M et al. Short-term effects of l-citrulline supplementation on arterial stiffness in middle-aged men. *Int J Cardiol* 2010 November 8.

(514) Cormio L, De SM, Lorusso F et al. Oral L-citrulline supplementation improves erection hardness in men with mild erectile dysfunction. *Urology* 2011 January;77(1):119-22.

(515) Tsuchiya K, Kanematsu Y, Yoshizumi M et al. Nitrite is an alternative source of NO in vivo. *Am J Physiol Heart Circ Physiol* 2005 May;288(5):H2163-H2170.

(516) Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov* 2008 February;7(2):156-67.

(517) Petersson J, Carlstrom M, Schreiber O et al. Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. *Free Radic Biol Med* 2009 April 15;46(8):1068-75.

(518) Webb AJ, Patel N, Loukogeorgakis S et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* 2008 March;51(3):784-90.

(519) Larsen FJ, Ekblom B, Sahlin K, Lundberg JO, Weitzberg E. Effects of dietary nitrate on blood pressure in healthy volunteers. *N Engl J Med* 2006 December 28;355(26):2792-3.

(520) Kapil V, Milsom AB, Okorie M et al. Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. *Hypertension* 2010 August;56(2):274-81.

(521) Presley TD, Morgan AR, Bechtold E et al. Acute effect of a high nitrate diet on brain perfusion in older adults. *Nitric Oxide* 2011 January 1;24(1):34-42.

(522) Pattillo CB, Bir S, Rajaram V, Kevil CG. Inorganic nitrite and chronic tissue ischaemia: a novel therapeutic modality for peripheral vascular diseases. *Cardiovasc Res* 2010 October 19.

(523) Bailey SJ, Winyard P, Vanhatalo A et al. Dietary nitrate supplementation reduces the O2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol* 2009 October;107(4):1144-55.

(524) Lansley KE, Winyard PG, Fulford J et al. Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study. *J Appl Physiol* 2011 March;110(3):591-600.

(525) Larsen FJ, Schiffer TA, Borniquel S et al. Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab* 2011 February 2;13(2):149-59.

(526) Kenjale AA, Ham KL, Stabler T et al. Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *J Appl Physiol* 2011 June;110(6):1582-91.

(527) Joshipura KJ, Hu FB, Manson JE et al. The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann Intern Med* 2001 June 19;134(12):1106-14.

(528) Joshipura KJ, Ascherio A, Manson JE et al. Fruit and vegetable intake in relation to risk of ischemic stroke. *JAMA* 1999 October 6;282(13):1233-9.

(529) Joshipura KJ, Hung HC, Li TY et al. Intakes of fruits, vegetables and carbohydrate and the risk of CVD. *Public Health Nutr* 2009 January;12(1):115-21.

(530) Lundberg JO, Feelisch M, Bjorne H, Jansson EA, Weitzberg E. Cardioprotective effects of vegetables: is nitrate the answer? *Nitric Oxide* 2006 December;15(4):359-62.

(531) Hung HC, Joshipura KJ, Jiang R et al. Fruit and vegetable intake and risk of major chronic disease. *J Natl Cancer Inst* 2004 November 3;96(21):1577-84.

(532) Montonen J, Jarvinen R, Heliovaara M, Reunanen A, Aromaa A, Knekt P. Food consumption and the incidence of type II diabetes mellitus. *Eur J Clin Nutr* 2005 March;59(3):441-8.

(533) Bazzano LA, Li TY, Joshipura KJ, Hu FB. Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. *Diabetes Care* 2008 July;31(7):1311-7.

(534) Nettleton JA, Steffen LM, Ni H, Liu K, Jacobs DR, Jr. Dietary patterns and risk of incident type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care* 2008 September;31(9):1777-82.

(535) Hawley SA, Ross FA, Chevtzoff C et al. Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab* 2010 June 9;11(6):554-65.

(536) Xiao B, Sanders MJ, Underwood E et al. Structure of mammalian AMPK and its regulation by ADP. *Nature* 2011 April 14;472(7342):230-3.

(537) Chen ZP, Mitchelhill KI, Michell BJ et al. AMP-activated protein kinase phosphorylation of endothelial NO synthase. *FEBS Lett* 1999 January 29;443(3):285-9.

(538) Chen Z, Peng IC, Sun W et al. AMP-activated protein kinase functionally phosphorylates endothelial nitric oxide synthase Ser633. *Circ Res* 2009 February 27;104(4):496-505.

(539) Zhou G, Myers R, Li Y et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001 October;108(8):1167-74.

(540) Musi N, Hirshman MF, Nygren J et al. Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. *Diabetes* 2002 July;51(7):2074-81.

(541) Lee YS, Kim WS, Kim KH et al. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes* 2006 August;55(8):2256-64.

(542) Turner N, Li JY, Gosby A et al. Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: a mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. *Diabetes* 2008 May;57(5):1414-8.

(543) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998 September 12;352(9131):854-65.

(544) Scarpello JH. Improving survival with metformin: the evidence base today. *Diabetes Metab* 2003 September;29(4 Pt 2):6S36-43.

(545) Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008 October 9;359(15):1577-89.

(546) Tzoulaki I, Molokhia M, Curcin V et al. Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database. *BMJ* 2009;339:b4731.

(547) Roussel R, Travert F, Pasquet B et al. Metformin use and mortality among patients with diabetes and atherothrombosis. *Arch Intern Med* 2010 November 22;170(21):1892-9.

(548) Schramm TK, Gislason GH, Vaag A et al. Mortality and cardiovascular risk associated with different insulin secretagogues compared with metformin in type 2 diabetes, with or without a previous myocardial infarction: a nationwide study. *Eur Heart J* 2011 August;32(15):1900-8.

(549) Canto C, Auwerx J. Calorie Restriction: Is AMPK a Key Sensor and Effector? *Physiology (Bethesda)* 2011 August;26(4):214-24.

(550) Canto C, Gerhart-Hines Z, Feige JN et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 2009 April 23;458(7241):1056-60.

(551) McCarty MF. Chronic activation of AMP-activated kinase as a strategy for slowing aging. *Med Hypotheses* 2004;63(2):334-9.

(552) Anisimov VN. Metformin for aging and cancer prevention. *Aging (Albany NY)* 2010 November;2(11):760-74.

(553) McCarty MF. AMPK activation - protean potential for boosting healthspan. *Medical Hypotheses* 2012;submitted for publication.

(554) Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ* 2005 June 4;330(7503):1304-5.

(555) Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 2009 September;32(9):1620-5.

(556) Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 2009 September;52(9):1766-77.

(557) Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care* 2010 February;33(2):322-6.

(558) Bowker SL, Yasui Y, Veugelers P, Johnson JA. Glucose-lowering agents and cancer mortality rates in type 2 diabetes: assessing effects of time-varying exposure. *Diabetologia* 2010 April 21.

(559) Decensi A, Puntoni M, Goodwin P et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. *Cancer Prev Res (Phila)* 2010 November;3(11):1451-61.

(560) Jiralerspong S, Palla SL, Giordano SH et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol* 2009 July 10;27(20):3297-302.

(561) Tan BX, Yao WX, Ge J et al. Prognostic influence of metformin as first-line chemotherapy for advanced nonsmall cell lung cancer in patients with type 2 diabetes. *Cancer* 2011 April 26.

(562) Lee JH, Kim TI, Jeon SM, Hong SP, Cheon JH, Kim WH. The effects of metformin on the survival of colorectal cancer patients with diabetes mellitus. *Int J Cancer* 2011 September 12.

(563) Dowling RJ, Goodwin PJ, Stambolic V. Understanding the benefit of metformin use in cancer treatment. *BMC Med* 2011;9:33.

(564) Egan D, Kim J, Shaw RJ, Guan KL. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. *Autophagy* 2011 June;7(6):643-4.

(565) Cuervo AM. Calorie restriction and aging: the ultimate "cleansing diet". *J Gerontol A Biol Sci Med Sci* 2008 June;63(6):547-9.

(566) Hariharan N, Maejima Y, Nakae J, Paik J, DePinho RA, Sadoshima J. Deacetylation of FoxO by Sirt1 Plays an Essential Role in Mediating Starvation-Induced Autophagy in Cardiac Myocytes. *Circ Res* 2010 December 10;107(12):1470-82.

(567) Canto C, Jiang LQ, Deshmukh AS et al. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metab* 2010 March 3;11(3):213-9.

(568) Kukidome D, Nishikawa T, Sonoda K et al. Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes* 2006 January;55(1):120-7.

(569) Alba G, El BR, varez-Maqueda M et al. Stimulators of AMP-activated protein kinase inhibit the respiratory burst in human neutrophils. *FEBS Lett* 2004 August 27;573(1-3):219-25.

(570) Piwkowska A, Rogacka D, Jankowski M, Dominiczak MH, Stepinski JK, Angielski S. Metformin induces suppression of NAD(P)H oxidase activity in podocytes. *Biochem Biophys Res Commun* 2010 March 5;393(2):268-73.

(571) Eid AA, Ford BM, Block K et al. AMP-activated protein kinase (AMPK) negatively regulates Nox4-dependent activation of p53 and epithelial cell apoptosis in diabetes. *J Biol Chem* 2010 November 26;285(48):37503-12.

(572) Schuhmacher S, Foretz M, Knorr M et al. alpha1AMP-activated protein kinase preserves endothelial function during chronic angiotensin II treatment by limiting Nox2 upregulation. *Arterioscler Thromb Vasc Biol* 2011 March;31(3):560-6.

(573) Colombo SL, Moncada S. AMPKalpha1 regulates the antioxidant status of vascular endothelial cells. *Biochem J* 2009 July 15;421(2):163-9.

(574) Greer EL, Oskoui PR, Banko MR et al. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J Biol Chem* 2007 October 12;282(41):30107-19.

(575) Olmos Y, Valle I, Borniquel S et al. Mutual dependence of Foxo3a and PGC-1alpha in the induction of oxidative stress genes. *J Biol Chem* 2009 May 22;284(21):14476-84.

(576) Algire C, Moiseeva O, schenes-Simard X et al. Metformin reduces endogenous reactive oxygen species and associated DNA damage. *Cancer Prev Res (Phila)* 2012 January 18.

(577) Wang Q, Zhang M, Liang B, Shirwany N, Zhu Y, Zou MH. Activation of AMP-activated protein kinase is required for berberine-induced reduction of atherosclerosis in mice: the role of uncoupling protein 2. *PLoS ONE* 2011;6(9):e25436.

(578) McCarty MF. Rationale for a novel nutraceutical complex 'K-water': potassium taurine bicarbonate (PTB). *Med Hypotheses* 2006;67(1):65-70.

(579) Cappuccio FP, MacGregor GA. Does potassium supplementation lower blood pressure? A meta-analysis of published trials. *J Hypertens JID - 8306882* 1991 May;9(5):465-73.

(580) Whelton PK, He J, Cutler JA et al. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. *JAMA JID - 7501160* 1997 May 28;277(20):1624-32.

(581) Khaw KT, Barrett-Connor E. Dietary potassium and stroke-associated mortality. A 12-year prospective population study. *N Engl J Med JID - 0255562* 1987 January 29;316(5):235-40.

(582) Fang J, Madhavan S, Alderman MH. Dietary potassium intake and stroke mortality. *Stroke JID - 0235266* 2000 July;31(7):1532-7.

(583) Iso H, Stampfer MJ, Manson JE et al. Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. *Stroke JID - 0235266* 1999 September;30(9):1772-9.

(584) Suter PM. The effects of potassium, magnesium, calcium, and fiber on risk of stroke. *Nutr Rev JID - 0376405* 1999 March;57(3):84-8.

(585) Sohn HY, Keller M, Gloe T, Morawietz H, Rueckschloss U, Pohl U. The small G-protein Rac mediates depolarization-induced superoxide formation in human endothelial cells. *J Biol Chem* 2000 June 23;275(25):18745-50.

(586) McCarty MF. Endothelial membrane potential regulates production of both nitric oxide and superoxide--a fundamental determinant of vascular health. *Med Hypotheses* 1999 October;53(4):277-89.

(587) Liu R, Garvin JL, Ren Y, Pagano PJ, Carretero OA. Depolarization of the macula densa induces superoxide production via NAD(P)H oxidase. *Am J Physiol Renal Physiol* 2007 June;292(6):F1867-F1872.

(588) Liu R, Juncos LA. GTPase-Rac enhances depolarization-induced superoxide production by the macula densa during tubuloglomerular feedback. *Am J Physiol Regul Integr Comp Physiol* 2010 February;298(2):R453-R458.

(589) McCabe RD, Bakarich MA, Srivastava K, Young DB. Potassium inhibits free radical formation. *Hypertension* 1994 July;24(1):77-82.

(590) Young DB, Lin H, McCabe RD. Potassium's cardiovascular protective mechanisms. *Am J Physiol* 1995 April;268(4 Pt 2):R825-R837.

(591) Yang BC, Li DY, Weng YF, Lynch J, Wingo CS, Mehta JL. Increased superoxide anion generation and altered vasoreactivity in rabbits on low-potassium diet. *Am J Physiol* 1998 June;274(6 Pt 2):H1955-H1961.

(592) Landmark K. [Hypokalemia can accelerate the development of cerebrovascular and cardiovascular disease]. *Tidsskr Nor Laegeforen* 2002 February 20;122(5):499-501.

(593) Raij L, Luscher TF, Vanhoutte PM. High potassium diet augments endothelium-dependent relaxations in the Dahl rat. *Hypertension* 1988 December;12(6):562-7.

(594) Sugimoto T, Tobian L, Ganguli MC. High potassium diets protect against dysfunction of endothelial cells in stroke-prone spontaneously hypertensive rats. *Hypertension* 1988 June;11(6 Pt 2):579-85.

(595) Zhou MS, Nishida Y, Yoneyama H, Chen QH, Kosaka H. Potassium supplementation increases sodium excretion and nitric oxide production in hypertensive Dahl rats. *Clin Exp Hypertens* 1999 November;21(8):1397-411.

(596) Zhou MS, Kosaka H, Yoneyama H. Potassium augments vascular relaxation mediated by nitric oxide in the carotid arteries of hypertensive Dahl rats. *Am J Hypertens* 2000 June;13(6 Pt 1):666-72.

(597) Fedorova OV, Talan MI, Agalakova NI, Lakatta EG, Bagrov AY. Endogenous ligand of alpha(1) sodium pump, marinobufagenin, is a novel mediator of sodium chloride--dependent hypertension. *Circulation* 2002 March 5;105(9):1122-7.

(598) Bagrov AY, Fedorova OV. Cardenolide and bufadienolide ligands of the sodium pump. How they work together in NaCl sensitive hypertension. *Front Biosci* 2005;10:2250-6.

(599) Zvartau NE, Conrady AO, Sviryaev YV et al. Marinobufagenin in hypertensive patients with obstructive sleep apnea. *Cell Mol Biol (Noisy -le-grand)* 2006;52(8):24-7.

(600) Fedorova OV, Shapiro JI, Bagrov AY. Endogenous cardiotonic steroids and salt-sensitive hypertension. *Biochim Biophys Acta* 2010 December;1802(12):1230-6.

(601) McCarty MF. Marinobufagenin may mediate the impact of salty diets on left ventricular hypertrophy by disrupting the protective function of coronary microvascular endothelium. *Med Hypotheses* 2005;64(4):854-63.

(602) Kennedy DJ, Vetteth S, Periyasamy SM et al. Central role for the cardiotonic steroid marinobufagenin in the pathogenesis of experimental uremic cardiomyopathy. *Hypertension* 2006 March;47(3):488-95.

(603) Danchuk S, Sukhanov S, Horvat D, Uddin MN, Puschett JB. Effects of resibufogenin in experimental hypertension. *Am J Nephrol* 2008;28(1):8-13.

(604) Dietz R. The role of potassium in hypertension. Discovery of potassium as a natriuretic and antihypertensive agent. *Am J Nephrol JID - 8109361* 1983 March;3(2-3):100-8.

(605) Smith SR, Klotman PE, Svetkey LP. Potassium chloride lowers blood pressure and causes natriuresis in older patients with hypertension. *J Am Soc Nephrol JID - 9013836* 1992 February;2(8):1302-9.

(606) Krishna GG, Kapoor SC. Potassium supplementation ameliorates mineralocorticoid-induced sodium retention. *Kidney Int JID - 0323470* 1993 May;43(5):1097-103.

(607) Antonios TF, MacGregor GA. Deleterious effects of salt intake other than effects on blood pressure. *Clin Exp Pharmacol Physiol* 1995 March;22(3):180-4.

(608) Boynton H, McCarty MF, Moore RD. *The Salt Solution*. New York: Avery; 2001.

(609) McCarty MF. Up-regulation of endothelial nitric oxide activity as a central strategy for prevention of ischemic stroke - just say NO to stroke! *Med Hypotheses* 2000 November;55(5):386-403.

(610) Chang HY, Hu YW, Yue CS et al. Effect of potassium-enriched salt on cardiovascular mortality and medical expenses of elderly men. *Am J Clin Nutr* 2006 June;83(6):1289-96.

(611) Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PW, Kiel DP. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. *Am J Clin Nutr JID - 0376027* 1999 April;69(4):727-36.

(612) New SA, Robins SP, Campbell MK et al. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? *Am J Clin Nutr JID - 0376027* 2000 January;71(1):142-51.

(613) Lemann JJ, Pleuss JA, Gray RW. Potassium causes calcium retention in healthy adults. *J Nutr JID - 0404243* 1993 September;123(9):1623-6.

(614) Sebastian A, Morris RCJ. Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. *N Engl J Med JID - 0255562* 1994 July 28;331(4):279-Sebastian, A.

(615) Frassetto L, Morris RC, Jr., Sebastian A. Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women. *J Clin Endocrinol Metab* 2005 February;90(2):831-4.

(616) Wachman A, Bernstein DS. Diet and osteoporosis. *Lancet* 1968 May 4;1(7549):958-9.

(617) Frassetto LA, Todd KM, Morris RCJ, Sebastian A. Worldwide incidence of hip fracture in elderly women: relation to consumption of animal and vegetable foods. *J Gerontol A Biol Sci Med Sci JID - 9502837* 2000 October;55(10):M585-M592.

(618) McCarty MF. Rationale for a novel nutraceutical complex 'K-water': potassium taurine bicarbonate (PTB). *Med Hypotheses* 2006;67(1):65-70.

(619) McCarty MF. Sub-optimal taurine status may promote platelet hyperaggregability in vegetarians. *Med Hypotheses* 2004;63(3):426-33.

(620) Azuma J, Sawamura A, Awata N et al. Therapeutic effect of taurine in congestive heart failure: a double-blind crossover trial. *Clin Cardiol* 1985 May;8(5):276-82.

(621) Azuma J. Long-term effect of taurine in congestive heart failure: preliminary report. Heart Failure Research with Taurine Group. *Adv Exp Med Biol* 1994;359:425-33.

(622) Takihara K, Azuma J, Awata N et al. Beneficial effect of taurine in rabbits with chronic congestive heart failure. *Am Heart J* 1986 December;112(6):1278-84.

(623) Elizarova EP, Orlova TR, Medvedeva NV. Effects on heart membranes after taurine treatment in rabbits with congestive heart failure. *Arzneimittelforschung* 1993 March;43(3):308-12.

(624) Hayes KC, Pronczuk A, Addesa AE, Stephan ZF. Taurine modulates platelet aggregation in cats and humans. *Am J Clin Nutr* 1989 June;49(6):1211-6.

(625) Franconi F, Bennardini F, Mattana A et al. Plasma and platelet taurine are reduced in subjects with insulin-dependent diabetes mellitus: effects of taurine supplementation. *Am J Clin Nutr* 1995 May;61(5):1115-9.

(626) Barua M, Liu Y, Quinn MR. Taurine chloramine inhibits inducible nitric oxide synthase and TNF-alpha gene expression in activated alveolar macrophages: decreased NF-kappaB activation and IkappaB kinase activity. *J Immunol* 2001 August 15;167(4):2275-81.

(627) Kanayama A, Inoue J, Sugita-Konishi Y, Shimizu M, Miyamoto Y. Oxidation of Ikappa Balpha at methionine 45 is one cause of taurine chloramine-induced inhibition of NF-kappa B activation. *J Biol Chem* 2002 July 5;277(27):24049-56.

(628) Schuller-Levis G, Quinn MR, Wright C, Park E. Taurine protects against oxidant-induced lung injury: possible mechanism(s) of action. *Adv Exp Med Biol* 1994;359:31-9.

(629) Sener G, Sehirli O, Ipci Y et al. Protective effects of taurine against nicotine-induced oxidative damage of rat urinary bladder and kidney. *Pharmacology* 2005 April;74(1):37-44.

(630) Zhao Z, Satsu H, Fujisawa M et al. Attenuation by dietary taurine of dextran sulfate sodium-induced colitis in mice and of THP-1-induced damage to intestinal Caco-2 cell monolayers. *Amino Acids* 2008 June;35(1):217-24.

(631) Schuller-Levis GB, Gordon RE, Wang C, Park E. Taurine reduces lung inflammation and fibrosis caused by bleomycin. *Adv Exp Med Biol* 2003;526:395-402.

(632) Di Leo MA, Santini SA, Silveri NG, Giardina B, Franconi F, Ghirlanda G. Long-term taurine supplementation reduces mortality rate in streptozotocin-induced diabetic rats. *Amino Acids* 2004 October;27(2):187-91.

(633) Franconi F, Loizzo A, Ghirlanda G, Seghieri G. Taurine supplementation and diabetes mellitus. *Curr Opin Clin Nutr Metab Care* 2006 January;9(1):32-6.

(634) Li F, Abatan OI, Kim H et al. Taurine reverses neurological and neurovascular deficits in Zucker diabetic fatty rats. *Neurobiol Dis* 2006 June;22(3):669-76.

(635) Yu X, Xu Z, Mi M et al. Dietary taurine supplementation ameliorates diabetic retinopathy via anti-excitotoxicity of glutamate in streptozotocin-induced Sprague-Dawley rats. *Neurochem Res* 2008 March;33(3):500-7.

(636) Higo S, Miyata S, Jiang QY, Kitazawa R, Kitazawa S, Kasuga M. Taurine administration after appearance of proteinuria retards progression of diabetic nephropathy in rats. *Kobe J Med Sci* 2008;54(1):E35-E45.

(637) Harada H, Kitazaki K, Tsujino T et al. Oral taurine supplementation prevents the development of ethanol-induced hypertension in rats. *Hypertens Res* 2000 May;23(3):277-84.

(638) Devi SL, Viswanathan P, Anuradha CV. Taurine enhances the metabolism and detoxification of ethanol and prevents hepatic fibrosis in rats treated with iron and alcohol. *Environ Toxicol Pharmacol* 2009 January;27(1):120-6.

(639) Chen X, Sebastian BM, Tang H et al. Taurine supplementation prevents ethanol-induced decrease in serum adiponectin and reduces hepatic steatosis in rats. *Hepatology* 2009 May;49(5):1554-62.

(640) Watanabe A, Hobara N, Nagashima H. Lowering of liver acetaldehyde but not ethanol concentrations by pretreatment with taurine in ethanol-loaded rats. *Experientia* 1985 November 15;41(11):1421-2.

(641) Watanabe A, Hobara N, Kobayashi M, Nakatsukasa H, Nagashima H. Lowering of blood acetaldehyde but not ethanol concentrations by pantethine following alcohol ingestion: different effects in flushing and nonflushing subjects. *Alcohol Clin Exp Res* 1985 May;9(3):272-6.

(642) Watanabe A, Hobara N, Kobayashi M. Effect of taurine on blood acetaldehyde elevation following alcohol ingestion. *Res Commun Subst Abuse* 1985;247-50.

(643) Guo R, Ren J. Alcohol and acetaldehyde in public health: from marvel to menace. *Int J Environ Res Public Health* 2010 April;7(4):1285-301.

(644) Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* 2007 August;7(8):599-612.

(645) Preedy VR, Crabb DW, Farres J, Emery PW. Alcoholic myopathy and acetaldehyde. *Novartis Found Symp* 2007;285:158-77.

(646) Lawler JM, Barnes WS, Wu G, Song W, Demaree S. Direct antioxidant properties of creatine. *Biochem Biophys Res Commun* 2002 January 11;290(1):47-52.

(647) Meyer LE, Machado LB, Santiago AP et al. Mitochondrial creatine kinase activity prevents reactive oxygen species generation: antioxidant role of mitochondrial kinase-dependent ADP re-cycling activity. *J Biol Chem* 2006 December 8;281(49):37361-71.

(648) Bemben MG, Lamont HS. Creatine supplementation and exercise performance: recent findings. *Sports Med* 2005;35(2):107-25.

(649) Kley RA, Vorgerd M, Tarnopolsky MA. Creatine for treating muscle disorders. *Cochrane Database Syst Rev* 2007;(1):CD004760.

(650) Gualano B, DE SP, V, Roschel H et al. Creatine in type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *Med Sci Sports Exerc* 2011 May;43(5):770-8.

(651) Alves CR, Ferreira JC, de Siqueira-Filho MA, Carvalho CR, Lancha AH, Jr., Gualano B. Creatine-induced glucose uptake in type 2 diabetes: a role for AMPK-alpha? *Amino Acids* 2012 October;43(4):1803-7.

(652) Deminice R, Jordao AA. Creatine supplementation reduces oxidative stress biomarkers after acute exercise in rats. *Amino Acids* 2012 August;43(2):709-15.

(653) Rahimi R. Creatine supplementation decreases oxidative DNA damage and lipid peroxidation induced by a single bout of resistance exercise. *J Strength Cond Res* 2011 December;25(12):3448-55.

(654) Guimaraes-Ferreira L, Pinheiro CH, Gerlinger-Romero F et al. Short-term creatine supplementation decreases reactive oxygen species content with no changes in expression and activity of antioxidant enzymes in skeletal muscle. *Eur J Appl Physiol* 2012 November;112(11):3905-11.

(655) Coco M, Perciavalle V. Creatine ingestion effects on oxidative stress in a steady-state test at 75% VO(2max). *J Sports Med Phys Fitness* 2012 April;52(2):165-9.

(656) Lygate CA, Bohl S, Ten HM et al. Moderate elevation of intracellular creatine by targeting the creatine transporter protects mice from acute myocardial infarction. *Cardiovasc Res* 2012 December 1;96(3):466-75.

(657) Carvalho AP, Rassi S, Fontana KE, Correa KS, Feitosa RH. Influence of creatine supplementation on the functional capacity of patients with heart failure. *Arq Bras Cardiol* 2012 July;99(1):623-9.

(658) Dechent P, Pouwels PJ, Wilken B, Hanefeld F, Frahm J. Increase of total creatine in human brain after oral supplementation of creatine-monohydrate. *Am J Physiol* 1999 September;277(3 Pt 2):R698-R704.

(659) Klein AM, Ferrante RJ. The neuroprotective role of creatine. *Subcell Biochem* 2007;46:205-43.

(660) Adhihetty PJ, Beal MF. Creatine and Its Potential Therapeutic Value for Targeting Cellular Energy Impairment in Neurodegenerative Diseases. *Neuromolecular Med* 2008 November 13.

(661) Yang L, Calingasan NY, Wille EJ et al. Combination therapy with coenzyme Q10 and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's diseases. *J Neurochem* 2009 June;109(5):1427-39.

(662) Bender A, Beckers J, Schneider I et al. Creatine improves health and survival of mice. *Neurobiol Aging* 2008 September;29(9):1404-11.

(663) Rae C, Digney AL, McEwan SR, Bates TC. Oral creatine monohydrate supplementation improves brain performance: a double-blind, placebo-controlled, cross-over trial. *Proc Biol Sci* 2003 October 22;270(1529):2147-50.

(664) Hammett ST, Wall MB, Edwards TC, Smith AT. Dietary supplementation of creatine monohydrate reduces the human fMRI BOLD signal. *Neurosci Lett* 2010 August 2;479(3):201-5.

(665) Benton D, Donohoe R. The influence of creatine supplementation on the cognitive functioning of vegetarians and omnivores. *Br J Nutr* 2011 April;105(7):1100-5.

(666) Stephens FB, Evans CE, Constantin-Teodosiu D, Greenhaff PL. Carbohydrate ingestion augments L-carnitine retention in humans. *J Appl Physiol* 2007 March;102(3):1065-70.

(667) Stephens FB, Constantin-Teodosiu D, Greenhaff PL. New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *J Physiol* 2007 June 1;581(Pt 2):431-44.

(668) McCarty MF. Orthomolecular aids for dieting. *Med Hypotheses* 1982 March;8(3):269-74.

(669) Malaguarnera M, Gargante MP, Russo C et al. L-carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis--a randomized and controlled clinical trial. *Am J Gastroenterol* 2010 June;105(6):1338-45.

(670) Ferrari R, Merli E, Cicchitelli G, Mele D, Fucili A, Ceconi C. Therapeutic effects of L-carnitine and propionyl-L-carnitine on cardiovascular diseases: a review. *Ann N Y Acad Sci* 2004 November;1033:79-91.

(671) Liu J, Head E, Gharib AM et al. Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or R-alpha -lipoic acid. *Proc Natl Acad Sci U S A* 2002 February 19;99(4):2356-61.

(672) Hagen TM, Moreau R, Suh JH, Visioli F. Mitochondrial decay in the aging rat heart: evidence for improvement by dietary supplementation with acetyl-L-carnitine and/or lipoic acid. *Ann N Y Acad Sci* 2002 April;959:491-507.

(673) Ames BN. Delaying the mitochondrial decay of aging. *Ann N Y Acad Sci* 2004 June;1019:406-11.

(674) McMackin CJ, Widlansky ME, Hamburg NM et al. Effect of combined treatment with alpha-Lipoic acid and acetyl-L-carnitine on vascular function and blood pressure in patients with coronary artery disease. *J Clin Hypertens (Greenwich)* 2007 April;9(4):249-55.

(675) Hagen TM, Liu J, Lykkesfeldt J et al. Feeding acetyl-L-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. *Proc Natl Acad Sci U S A* 2002 February 19;99(4):1870-5.

(676) Sener G, Paskaloglu K, Satiroglu H, Alican I, Kacmaz A, Sakarcan A. L-carnitine ameliorates oxidative damage due to chronic renal failure in rats. *J Cardiovasc Pharmacol* 2004 May;43(5):698-705.

(677) Sachan DS, Hongu N, Johnsen M. Decreasing oxidative stress with choline and carnitine in women. *J Am Coll Nutr* 2005 June;24(3):172-6.

(678) Garcia CL, Filippi S, Mosesso P et al. The protective effect of L-carnitine in peripheral blood human lymphocytes exposed to oxidative agents. *Mutagenesis* 2006 January;21(1):21-7.

(679) Calo LA, Pagnin E, Davis PA et al. Antioxidant effect of L-carnitine and its short chain esters: relevance for the protection from oxidative stress related cardiovascular damage. *Int J Cardiol* 2006 February 8;107(1):54-60.

(680) Gomez-Amores L, Mate A, Miguel-Carrasco JL et al. L-carnitine attenuates oxidative stress in hypertensive rats. *J Nutr Biochem* 2007 August;18(8):533-40.

(681) Chowdhury P, Soulsby M, Kim K. L-carnitine influence on oxidative stress induced by hind limb unloading in adult rats. *Aviat Space Environ Med* 2007 June;78(6):554-6.

(682) Shamsi FA, Chaudhry IA, Boulton ME, Al-Rajhi AA. L-carnitine protects human retinal pigment epithelial cells from oxidative damage. *Curr Eye Res* 2007 June;32(6):575-84.

(683) Thangasamy T, Jeyakumar P, Sittadjody S, Joyee AG, Chinnakannu P. L-carnitine mediates protection against DNA damage in lymphocytes of aged rats. *Biogerontology* 2009 April;10(2):163-72.

(684) Malaguarnera M, Vacante M, Avitabile T, Malaguarnera M, Cammalleri L, Motta M. L-Carnitine supplementation reduces oxidized LDL cholesterol in patients with diabetes. *Am J Clin Nutr* 2009 January;89(1):71-6.

(685) Saluk-Juszczak J, Olas B, Wachowicz B, Glowacki R, Bald E. L-carnitine modulates blood platelet oxidative stress. *Cell Biol Toxicol* 2010 August;26(4):355-65.

(686) Fatouros IG, Douroudos I, Panagoutsos S et al. Effects of L-carnitine on oxidative stress responses in patients with renal disease. *Med Sci Sports Exerc* 2010 October;42(10):1809-18.

(687) Mate A, Miguel-Carrasco JL, Monserrat MT, Vazquez CM. Systemic antioxidant properties of L-carnitine in two different models of arterial hypertension. *J Physiol Biochem* 2010 June;66(2):127-36.

(688) Listenberger LL, Han X, Lewis SE et al. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A* 2003 March 18;100(6):3077-82.

(689) Weinberg JM. Lipotoxicity. *Kidney Int* 2006 November;70(9):1560-6.

(690) Shankar SS, Mirzamohammadi B, Walsh JP, Steinberg HO. L-carnitine may attenuate free fatty acid-induced endothelial dysfunction. *Ann N Y Acad Sci* 2004 November;1033:189-97.

(691) Volek JS, Judelson DA, Silvestre R et al. Effects of carnitine supplementation on flow-mediated dilation and vascular inflammatory responses to a high-fat meal in healthy young adults. *Am J Cardiol* 2008 November 15;102(10):1413-7.

(692) Evans JD, Jacobs TF, Evans EW. Role of acetyl-L-carnitine in the treatment of diabetic peripheral neuropathy. *Ann Pharmacother* 2008 November;42(11):1686-91.

(693) Sayed-Ahmed MM, Khattab MM, Gad MZ, Mostafa N. L-carnitine prevents the progression of atherosclerotic lesions in hypercholesterolaemic rabbits. *Pharmacol Res* 2001 September;44(3):235-42.

(694) Pistone G, Marino A, Leotta C, Dell'Arte S, Finocchiaro G, Malaguarnera M. Levocarnitine administration in elderly subjects with rapid muscle fatigue: effect on body composition, lipid profile and fatigue. *Drugs Aging* 2003;20(10):761-7.

(695) Malaguarnera M, Cammalleri L, Gargante MP, Vacante M, Colonna V, Motta M. L-Carnitine treatment reduces severity of physical and mental fatigue and increases cognitive functions in centenarians: a randomized and controlled clinical trial. *Am J Clin Nutr* 2007 December;86(6):1738-44.

(696) Lynch JW, Han NI, Schofield PR. Building new function into glycine receptors: a structural model for the activation of the glycine-gated chloride channel. *Clin Exp Pharmacol Physiol* 1999 November;26(11):932-4.

(697) Gundersen RY, Vaagenes P, Breivik T, Fonnum F, Opstad PK. Glycine--an important neurotransmitter and cytoprotective agent. *Acta Anaesthesiol Scand* 2005 September;49(8):1108-16.

(698) Froh M, Thurman RG, Wheeler MD. Molecular evidence for a glycine-gated chloride channel in macrophages and leukocytes. *Am J Physiol Gastrointest Liver Physiol* 2002 October;283(4):G856-G863.

(699) Yamashina S, Konno A, Wheeler MD et al. Endothelial cells contain a glycine-gated chloride channel. *Nutr Cancer* 2001;40(2):197-204.

(700) Wheeler M, Stachlewitz RF, Yamashina S, Ikejima K, Morrow AL, Thurman RG. Glycine-gated chloride channels in neutrophils attenuate calcium influx and superoxide production. *FASEB J* 2000 March;14(3):476-84.

(701) Wheeler MD, Thurman RG. Production of superoxide and TNF-alpha from alveolar macrophages is blunted by glycine. *Am J Physiol* 1999 November;277(5 Pt 1):L952-L959.

(702) Wheeler MD, Ikejema K, Enomoto N et al. Glycine: a new anti-inflammatory immunonutrient. *Cell Mol Life Sci* 1999 November 30;56(9-10):843-56.

(703) Li X, Bradford BU, Wheeler MD et al. Dietary glycine prevents peptidoglycan polysaccharide-induced reactive arthritis in the rat: role for glycine-gated chloride channel. *Infect Immun* 2001 September;69(9):5883-91.

(704) Yamashina S, Ikejima K, Enomoto N, Takei Y, Sato N. Glycine as a therapeutic immuno-nutrient for alcoholic liver disease. *Alcohol Clin Exp Res* 2005 November;29(11 Suppl):162S-5S.

(705) Wheeler MD, Rose ML, Yamashima S et al. Dietary glycine blunts lung inflammatory cell influx following acute endotoxin. *Am J Physiol Lung Cell Mol Physiol* 2000 August;279(2):L390-L398.

(706) Zhong Z, Wheeler MD, Li X et al. L-Glycine: a novel antiinflammatory, immunomodulatory, and cytoprotective agent. *Curr Opin Clin Nutr Metab Care* 2003 March;6(2):229-40.

(707) Senthilkumar R, Viswanathan P, Nalini N. Glycine modulates hepatic lipid accumulation in alcohol-induced liver injury. *Pol J Pharmacol* 2003 July;55(4):603-11.

(708) Senthilkumar R, Viswanathan P, Nalini N. Effect of glycine on oxidative stress in rats with alcohol induced liver injury. *Pharmazie* 2004 January;59(1):55-60.

(709) Senthilkumar R, Nalini N. Glycine prevents hepatic fibrosis by preventing the accumulation of collagen in rats with alcoholic liver injury. *Pol J Pharmacol* 2004 January;56(1):121-8.

(710) Senthilkumar R, Nalini N. Effect of glycine on tissue fatty acid composition in an experimental model of alcohol-induced hepatotoxicity. *Clin Exp Pharmacol Physiol* 2004 July;31(7):456-61.

(711) Nath KA, Ngo EO, Hebbel RP, Croatt AJ, Zhou B, Nutter LM. alpha-Ketoacids scavenge H2O2 in vitro and in vivo and reduce menadione-induced DNA injury and cytotoxicity. *Am J Physiol* 1995 January;268(1 Pt 1):C227-C236.

(712) Das UN. Is pyruvate an endogenous anti-inflammatory molecule? *Nutrition* 2006 September;22(9):965-72.

(713) Rose ML, Madren J, Bunzendahl H, Thurman RG. Dietary glycine inhibits the growth of B16 melanoma tumors in mice. *Carcinogenesis* 1999 May;20(5):793-8.

(714) Amin K, Li J, Chao WR, Dewhirst MW, Haroon ZA. Dietary glycine inhibits angiogenesis during wound healing and tumor growth. *Cancer Biol Ther* 2003 March;2(2):173-8.

(715) McCarty MF, Barroso-Aranda J, Contreras F. The hyperpolarizing impact of glycine on endothelial cells may be anti-atherogenic. *Med Hypotheses* 2009 February 16.

(716) Hafidi ME, Perez I, Zamora J, Soto V, Carvajal-Sandoval G, Banos G. Glycine intake decreases plasma free fatty acids, adipose cell size, and blood pressure in sucrose-fed rats. *Am J Physiol Regul Integr Comp Physiol* 2004 December;287(6):R1387-R1393.

(717) Gannon MC, Nuttall JA, Nuttall FQ. The metabolic response to ingested glycine. *Am J Clin Nutr* 2002 December;76(6):1302-7.

(718) Carvajal SG, Medina SR, Juarez E, RamosMartinez G, Carvajal Juarez ME. Effect of glycine on hemoglobin glycation in diabetic patients. *Proc West Pharmacol Soc* 1999;42:31-2.

(719) Carvajal SG, Juarez E, Ramos MG, Carvajal Juarez ME, Medina-Santillan R. Inhibition of hemoglobin glycation with glycine in induced diabetes mellitus in rats. *Proc West Pharmacol Soc* 1999;42:35-6.

(720) varado-Vasquez N, Lascurain R, Ceron E et al. Oral glycine administration attenuates diabetic complications in streptozotocin-induced diabetic rats. *Life Sci* 2006 June 13;79(3):225-32.

(721) Lezcano MD, Teran OL, Carvajal SG, Gutierrez de la CM, Teran ED, Estrada PS. Effect of glycine on the immune response of the experimentally diabetic rats. *Rev Alerg Mex* 2006 November;53(6):212-6.

(722) Cruz M, Maldonado-Bernal C, Mondragon-Gonzalez R et al. Glycine treatment decreases proinflammatory cytokines and increases interferon-gamma in patients with type 2 diabetes. *J Endocrinol Invest* 2008 August;31(8):694-9.

(723) Fernandez-Rodriguez CM, Gutierrez ML, Serrano PL et al. Factors influencing the rate of fibrosis progression in chronic hepatitis C. *Dig Dis Sci* 2004 November;49(11-12):1971-6.

(724) Kimura F, Hayashi H, Yano M et al. Additional effect of low iron diet on iron reduction therapy by phlebotomy for chronic hepatitis C. *Hepatogastroenterology* 2005 March;52(62):563-6.

(725) Tanaka N, Horiuchi A, Yamaura T, Komatsu M, Tanaka E, Kiyosawa K. Efficacy and safety of 6-month iron reduction therapy in patients with hepatitis C virus-related cirrhosis: a pilot study. *J Gastroenterol* 2007 January;42(1):49-55.

(726) Desai TK, Jamil LH, Balasubramaniam M, Koff R, Bonkovsky HL. Phlebotomy improves therapeutic response to interferon in patients with chronic hepatitis C: a meta-analysis of six prospective randomized controlled trials. *Dig Dis Sci* 2008 March;53(3):815-22.

(727) Kato J, Miyanishi K, Kobune M et al. Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. *J Gastroenterol* 2007 October;42(10):830-6.

(728) Alexander D, Ball MJ, Mann J. Nutrient intake and haematological status of vegetarians and age-sex matched omnivores. *Eur J Clin Nutr* 1994 August;48(8):538-46.

(729) Wilson AK, Ball MJ. Nutrient intake and iron status of Australian male vegetarians. *Eur J Clin Nutr* 1999 March;53(3):189-94.

(730) Fleming DJ, Tucker KL, Jacques PF, Dallal GE, Wilson PW, Wood RJ. Dietary factors associated with the risk of high iron stores in the elderly Framingham Heart Study cohort. *Am J Clin Nutr* 2002 December;76(6):1375-84.

(731) Haap M, Fritsche A, Mensing HJ, Haring HU, Stumvoll M. Association of high serum ferritin concentration with glucose intolerance and insulin resistance in healthy people. *Ann Intern Med* 2003 November 18;139(10):869-71.

(732) Wrede CE, Buettner R, Bollheimer LC, Scholmerich J, Palitzsch KD, Hellerbrand C. Association between serum ferritin and the insulin resistance syndrome in a representative population. *Eur J Endocrinol* 2006 February;154(2):333-40.

(733) Fumeron F, Pean F, Driss F et al. Ferritin and transferrin are both predictive of the onset of hyperglycemia in men and women over 3 years: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. *Diabetes Care* 2006 September;29(9):2090-4.

(734) Tsimihodimos V, Gazi I, Kalaitzidis R, Elisaf M, Siamopoulos KC. Increased serum ferritin concentrations and liver enzyme activities in patients with metabolic syndrome. *Metab Syndr Relat Disord* 2006;4(3):196-203.

(735) Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA* 2004 February 11;291(6):711-7.

(736) Jehn ML, Guallar E, Clark JM et al. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol* 2007 May 1;165(9):1047-54.

(737) McCarty MF. Hyperinsulinemia may boost both hematocrit and iron absorption by up-regulating activity of hypoxia-inducible factor-1alpha. *Med Hypotheses* 2003 November;61(5-6):567-73.

(738) Jiang R, Ma J, Ascherio A, Stampfer MJ, Willett WC, Hu FB. Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. *Am J Clin Nutr* 2004 January;79(1):70-5.

(739) Fernandez-Real JM, Penarroja G, Castro A, Garcia-Bragado F, Hernandez-Aguado I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. *Diabetes* 2002 April;51(4):1000-4.

(740) Loeb LA, James EA, Waltersdorph AM, Klebanoff SJ. Mutagenesis by the autoxidation of iron with isolated DNA. *Proc Natl Acad Sci U S A* 1988 June;85(11):3918-22.

(741) Toyokuni S. Role of iron in carcinogenesis: Cancer as a ferrotoxic disease. *Cancer Sci* 2008 October 23.

(742) Nakano M, Kawanishi Y, Kamohara S et al. Oxidative DNA damage (8-hydroxydeoxyguanosine) and body iron status: a study on 2507 healthy people. *Free Radic Biol Med* 2003 October 1;35(7):826-32.

(743) Tuomainen TP, Loft S, Nyyssonen K, Punnonen K, Salonen JT, Poulsen HE. Body iron is a contributor to oxidative damage of DNA. *Free Radic Res* 2007 March;41(3):324-8.

(744) Hori A, Mizoue T, Kasai H et al. Body iron store as a predictor of oxidative DNA damage in healthy men and women. *Cancer Sci* 2010 February;101(2):517-22.

(745) Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta* 2004 January;339(1-2):1-9.

(746) Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2009 April;27(2):120-39.

(747) Stevens RG, Graubard BI, Micozzi MS, Neriishi K, Blumberg BS. Moderate elevation of body iron level and increased risk of cancer occurrence and death. *Int J Cancer* 1994 February 1;56(3):364-9.

(748) Knekt P, Reunanen A, Takkunen H, Aromaa A, Heliovaara M, Hakulinen T. Body iron stores and risk of cancer. *Int J Cancer* 1994 February 1;56(3):379-82.

(749) Stevens RG, Jones DY, Micozzi MS, Taylor PR. Body iron stores and the risk of cancer. *N Engl J Med* 1988 October 20;319(16):1047-52.

(750) Selby JV, Friedman GD. Epidemiologic evidence of an association between body iron stores and risk of cancer. *Int J Cancer* 1988 May 15;41(5):677-82.

(751) Nelson RL. Iron and colorectal cancer risk: human studies. *Nutr Rev* 2001 May;59(5):140-8.

(752) Merk K, Mattsson B, Mattsson A, Holm G, Gullbring B, Bjorkholm M. The incidence of cancer among blood donors. *Int J Epidemiol* 1990 September;19(3):505-9.

(753) Edgren G, Reilly M, Hjalgrim H et al. Donation frequency, iron loss, and risk of cancer among blood donors. *J Natl Cancer Inst* 2008 April 16;100(8):572-9.

(754) Zacharski LR, Chow BK, Howes PS et al. Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial. *JAMA* 2007 February 14;297(6):603-10.

(755) Zacharski LR, Chow BK, Howes PS et al. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: results from a randomized trial. *J Natl Cancer Inst* 2008 July 16;100(14):996-1002.

(756) Bannon DI, Abounader R, Lees PS, Bressler JP. Effect of DMT1 knockdown on iron, cadmium, and lead uptake in Caco-2 cells. *Am J Physiol Cell Physiol* 2003 January;284(1):C44-C50.

(757) Lee BK, Kim Y. Iron deficiency is associated with increased levels of blood cadmium in the Korean general population: analysis of 2008-2009 Korean National Health and Nutrition Examination Survey data. *Environ Res* 2012 January;112:155-63.

(758) Gilca M, Stoian I, Atanasiu V, Virgolici B. The oxidative hypothesis of senescence. *J Postgrad Med* 2007 July;53(3):207-13.

(759) Lass A, Sohal BH, Weindruch R, Forster MJ, Sohal RS. Caloric restriction prevents age-associated accrual of oxidative damage to mouse skeletal muscle mitochondria. *Free Radic Biol Med* 1998 December;25(9):1089-97.

(760) Merry BJ. Oxidative stress and mitochondrial function with aging--the effects of calorie restriction. *Aging Cell* 2004 February;3(1):7-12.

(761) de Grey AD. A proposed mechanism for the lowering of mitochondrial electron leak by caloric restriction. *Mitochondrion* 2001 August;1(2):129-39.

(762) Descamps O, Riondel J, Ducros V, Roussel AM. Mitochondrial production of reactive oxygen species and incidence of age-associated lymphoma in OF1 mice: effect of alternate-day fasting. *Mech Ageing Dev* 2005 November;126(11):1185-91.

(763) Sanz A, Caro P, Ayala V, Portero-Otin M, Pamplona R, Barja G. Methionine restriction decreases mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins. *FASEB J* 2006 June;20(8):1064-73.

(764) Naudi A, Caro P, Jove M et al. Methionine restriction decreases endogenous oxidative molecular damage and increases mitochondrial biogenesis and uncoupling protein 4 in rat brain. *Rejuvenation Res* 2007 December;10(4):473-84.

(765) McCarty MF, Barroso-Aranda J, Contreras F. The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy. *Med Hypotheses* 2008 September 11.

(766) Stone JJ. 2010.
Ref Type: Personal Communication

(767) Johnson JB, Laub DR, John S. The effect on health of alternate day calorie restriction: eating less and more than needed on alternate days prolongs life. *Med Hypotheses* 2006;67(2):209-11.

(768) Johnson JB, Summer W, Cutler RG et al. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. *Free Radic Biol Med* 2007 March 1;42(5):665-74.

(769) Heller RF, Heller RF. Hyperinsulinemic obesity and carbohydrate addiction: the missing link is the carbohydrate frequency factor. *Med Hypotheses* 1994 May;42(5):307-12.

(770) Mattson MP. The need for controlled studies of the effects of meal frequency on health. *Lancet* 2005 June 4;365(9475):1978-80.

(771) Allen NE, Appleby PN, Davey GK, Key TJ. Hormones and diet: low insulin-like growth factor-I but normal bioavailable androgens in vegan men. *Br J Cancer* 2000 July;83(1):95-7.

(772) McCarty MF. Hepatic monitoring of essential amino acid availability may regulate IGF-I activity, thermogenesis, and fatty acid oxidation/synthesis. *Med Hypotheses* 2001 February;56(2):220-4.

(773) Allen NE, Appleby PN, Davey GK, Key TJ, Rinaldi S, Kaaks R. The effect of diet on serum insulin-like growth-factor-I and its main binding proteins. *IARC Sci Publ* 2002;156:295-6.

(774) Allen NE, Appleby PN, Davey GK, Kaaks R, Rinaldi S, Key TJ. The associations of diet with serum insulin-like growth factor I and its main binding proteins in 292 women meat-eaters, vegetarians, and vegans. *Cancer Epidemiol Biomarkers Prev* 2002 November;11(11):1441-8.

(775) Fontana L, Klein S, Holloszy JO. Long-term low-protein, low-calorie diet and endurance exercise modulate metabolic factors associated with cancer risk. *Am J Clin Nutr* 2006 December;84(6):1456-62.

(776) Fontana L, Weiss EP, Villareal DT, Klein S, Holloszy JO. Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. *Aging Cell* 2008 October;7(5):681-7.

(777) McCarty MF. Dietary saturate/unsaturate ratio as a determinant of adiposity. *Med Hypotheses* 2010 July;75(1):14-6.

(778) Lee PD, Conover CA, Powell DR. Regulation and function of insulin-like growth factor-binding protein-1. *Proc Soc Exp Biol Med* 1993 October;204(1):4-29.

(779) Frystyk J, Hussain M, Skjaerbaek C et al. Serum free IGF-I during a hyperinsulinemic clamp following 3 days of administration of IGF-I vs. saline. *Am J Physiol* 1997 September;273(3 Pt 1):E507-E513.

(780) McCarty MF. Insulin and IGF-I as determinants of low "Western" cancer rates in the rural third world. *Int J Epidemiol* 2004 August;33(4):908-10.

(781) Campbell TC. Dietary protein, growth factors, and cancer. *Am J Clin Nutr* 2007 June;85(6):1667.

(782) Murphy CT. The search for DAF-16/FOXO transcriptional targets: approaches and discoveries. *Exp Gerontol* 2006 October;41(10):910-21.

(783) Morris BJ. A forkhead in the road to longevity: the molecular basis of lifespan becomes clearer. *J Hypertens* 2005 July;23(7):1285-309.

(784) Kops GJ, Dansen TB, Polderman PE et al. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 2002 September 19;419(6904):316-21.

(785) Chiribau CB, Cheng L, Cucoranu IC, Yu YS, Clempus RE, Sorescu D. FOXO3A regulates peroxiredoxin III expression in human cardiac fibroblasts. *J Biol Chem* 2008 March 28;283(13):8211-7.

(786) Li M, Chiu JF, Gagne J, Fukagawa NK. Age-related differences in insulin-like growth factor-1 receptor signaling regulates Akt/FOXO3a and ERK/Fos pathways in vascular smooth muscle cells. *J Cell Physiol* 2008 November;217(2):377-87.

(787) Tan WQ, Wang K, Lv DY, Li PF. Foxo3a inhibits cardiomyocyte hypertrophy through transactivating catalase. *J Biol Chem* 2008 October 31;283(44):29730-9.

(788) Olmos Y, Valle I, Borniquel S et al. Mutual dependence of Foxo3a and PGC-1alpha in the induction of oxidative stress genes. *J Biol Chem* 2009 May 22;284(21):14476-84.

(789) Caro P, Gomez J, Sanchez I et al. Forty percent methionine restriction decreases mitochondrial oxygen radical production and leak at complex I during forward electron flow and lowers oxidative damage to proteins and mitochondrial DNA in rat kidney and brain mitochondria. *Rejuvenation Res* 2009 December;12(6):421-34.

(790) Liu J, Killilea DW, Ames BN. Age-associated mitochondrial oxidative decay: improvement of carnitine acetyltransferase substrate-binding affinity and activity in brain by feeding old rats acetyl-L- carnitine and/or R-alpha -lipoic acid. *Proc Natl Acad Sci U S A* 2002 February 19;99(4):1876-81.

(791) Liu J, Atamna H, Kuratsune H, Ames BN. Delaying brain mitochondrial decay and aging with mitochondrial antioxidants and metabolites. *Ann N Y Acad Sci* 2002 April;959:133-66.

(792) Hagen TM, Moreau R, Suh JH, Visioli F. Mitochondrial decay in the aging rat heart: evidence for improvement by dietary supplementation with acetyl-L-carnitine and/or lipoic acid. *Ann N Y Acad Sci* 2002 April;959:491-507.

(793) Shen W, Liu K, Tian C et al. R-alpha-lipoic acid and acetyl-L-carnitine complementarily promote mitochondrial biogenesis in murine 3T3-L1 adipocytes. *Diabetologia* 2008 January;51(1):165-74.

(794) Long J, Gao F, Tong L, Cotman CW, Ames BN, Liu J. Mitochondrial decay in the brains of old rats: ameliorating effect of alpha-lipoic acid and acetyl-L-carnitine. *Neurochem Res* 2009 April;34(4):755-63.

(795) Cassano P, Sciancalepore AG, Pesce V et al. Acetyl-L-carnitine feeding to unloaded rats triggers in soleus muscle the coordinated expression of genes involved in mitochondrial biogenesis. *Biochim Biophys Acta* 2006 September;1757(9-10):1421-8.

(796) McCarty MF, Barroso-Aranda J, Contreras F. The "rejuvenatory" impact of lipoic acid on mitochondrial function in aging rats may reflect induction and activation of PPAR-gamma coactivator-1alpha. *Med Hypotheses* 2009 January;72(1):29-33.

(797) Wang Y, Li X, Guo Y, Chan L, Guan X. alpha-Lipoic acid increases energy expenditure by enhancing adenosine monophosphate-activated protein kinase-peroxisome proliferator-activated receptor-gamma coactivator-1alpha signaling in the skeletal muscle of aged mice. *Metabolism* 2010 July;59(7):967-76.

(798) Pesce V, Fracasso F, Cassano P, Lezza AM, Cantatore P, Gadaleta MN. Acetyl-L-carnitine supplementation to old rats partially reverts the age-related mitochondrial decay of soleus muscle by activating peroxisome proliferator-activated receptor gamma coactivator-1alpha-dependent mitochondrial biogenesis. *Rejuvenation Res* 2010 April;13(2-3):148-51.

(799) Shenk JC, Liu J, Fischbach K et al. The effect of acetyl-L-carnitine and R-alpha-lipoic acid treatment in ApoE4 mouse as a model of human Alzheimer's disease. *J Neurol Sci* 2009 August 15;283(1-2):199-206.

(800) McMackin CJ, Widlansky ME, Hamburg NM et al. Effect of combined treatment with alpha-Lipoic acid and acetyl-L-carnitine on vascular function and blood pressure in patients with coronary artery disease. *J Clin Hypertens (Greenwich)* 2007 April;9(4):249-55.

(801) Cioffi F, Senese R, de LP, Goglia F, Lanni A, Lombardi A. Uncoupling proteins: a complex journey to function discovery. *Biofactors* 2009 September;35(5):417-28.

(802) Azzu V, Brand MD. The on-off switches of the mitochondrial uncoupling proteins. *Trends Biochem Sci* 2010 May;35(5):298-307.

(803) Tainter ML, Stockton AB, Cutting WC. Use of dinitrophenol in obesity and related conditions: a progress report. *JAMA* 1933;101:1472-5.

(804) Colman E. Dinitrophenol and obesity: an early twentieth-century regulatory dilemma. *Regul Toxicol Pharmacol* 2007 July;48(2):115-7.

(805) Miranda EJ, McIntyre IM, Parker DR, Gary RD, Logan BK. Two deaths attributed to the use of 2,4-dinitrophenol. *J Anal Toxicol* 2006 April;30(3):219-22.

(806) Siegmueller C, Narasimhaiah R. 'Fatal 2,4-dinitrophenol poisoning... coming to a hospital near you'. *Emerg Med J* 2010 August;27(8):639-40.

(807) McFee RB, Caraccio TR, McGuigan MA, Reynolds SA, Bellanger P. Dying to be thin: a dinitrophenol related fatality. *Vet Hum Toxicol* 2004 October;46(5):251-4.

(808) Barros MH, Bandy B, Tahara EB, Kowaltowski AJ. Higher respiratory activity decreases mitochondrial reactive oxygen release and increases life span in Saccharomyces cerevisiae. *J Biol Chem* 2004 November 26;279(48):49883-8.

(809) Padalko VI. Uncoupler of oxidative phosphorylation prolongs the lifespan of Drosophila. *Biochemistry (Mosc)* 2005 September;70(9):986-9.

(810) Caldeira da Silva CC, Cerqueira FM, Barbosa LF, Medeiros MH, Kowaltowski AJ. Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance and longevity. *Aging Cell* 2008 August;7(4):552-60.

(811) O'Donnell RW, Johnson DK, Ziegler LM, DiMattina AJ, Stone RI, Holland JA. Endothelial NADPH oxidase: mechanism of activation by low-density lipoprotein. *Endothelium* 2003;10(6):291-7.

(812) Galle J, Hansen-Hagge T, Wanner C, Seibold S. Impact of oxidized low density lipoprotein on vascular cells. *Atherosclerosis* 2006 April;185(2):219-26.

(813) Stielow C, Catar RA, Muller G et al. Novel Nox inhibitor of oxLDL-induced reactive oxygen species formation in human endothelial cells. *Biochem Biophys Res Commun* 2006 May 26;344(1):200-5.

(814) Qamirani E, Ren Y, Kuo L, Hein TW. C-reactive protein inhibits endothelium-dependent NO-mediated dilation in coronary arterioles by activating p38 kinase and NAD(P)H oxidase. *Arterioscler Thromb Vasc Biol* 2005 May;25(5):995-1001.

(815) Nagaoka T, Kuo L, Ren Y, Yoshida A, Hein TW. C-reactive protein inhibits endothelium-dependent nitric oxide-mediated dilation of retinal arterioles via enhanced superoxide production. *Invest Ophthalmol Vis Sci* 2008 May;49(5):2053-60.

(816) Closhen D, Bender B, Luhmann HJ, Kuhlmann CR. CRP-induced levels of oxidative stress are higher in brain than aortic endothelial cells. *Cytokine* 2010 May;50(2):117-20.

(817) Howard AB, Alexander RW, Nerem RM, Griendling KK, Taylor WR. Cyclic strain induces an oxidative stress in endothelial cells. *Am J Physiol* 1997 February;272(2 Pt 1):C421-C427.

(818) Matsushita H, Lee KH, Tsao PS. Cyclic strain induces reactive oxygen species production via an endothelial NAD(P)H oxidase. *J Cell Biochem Suppl* 2001;Suppl 36:99-106.

(819) Inoguchi T, Sonta T, Tsubouchi H et al. Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: role of vascular NAD(P)H oxidase. *J Am Soc Nephrol* 2003 August;14(8 Suppl 3):S227-S232.

(820) Nakagami H, Kaneda Y, Ogihara T, Morishita R. Endothelial dysfunction in hyperglycemia as a trigger of atherosclerosis. *Curr Diabetes Rev* 2005 February;1(1):59-63.

(821) Fatehi-Hassanabad Z, Chan CB, Furman BL. Reactive oxygen species and endothelial function in diabetes. *Eur J Pharmacol* 2010 June 25;636(1-3):8-17.

(822) Inoguchi T, Li P, Umeda F et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* 2000 November;49(11):1939-45.

(823) Chinen I, Shimabukuro M, Yamakawa K et al. Vascular lipotoxicity: endothelial dysfunction via fatty-acid-induced reactive oxygen species overproduction in obese Zucker diabetic fatty rats. *Endocrinology* 2007 January;148(1):160-5.

(824) Dong F, Zhang X, Li SY et al. Possible involvement of NADPH oxidase and JNK in homocysteine-induced oxidative stress and apoptosis in human umbilical vein endothelial cells. *Cardiovasc Toxicol* 2005;5(1):9-20.

(825) Tyagi N, Sedoris KC, Steed M, Ovechkin AV, Moshal KS, Tyagi SC. Mechanisms of homocysteine-induced oxidative stress. *Am J Physiol Heart Circ Physiol* 2005 December;289(6):H2649-H2656.

(826) Edirimanne VE, Woo CW, Siow YL, Pierce GN, Xie JY, O K. Homocysteine stimulates NADPH oxidase-mediated superoxide production leading to endothelial dysfunction in rats. *Can J Physiol Pharmacol* 2007 December;85(12):1236-47.

(827) Bayorh MA, Ganafa AA, Socci RR, Silvestrov N, Abukhalaf IK. The role of oxidative stress in salt-induced hypertension. *Am J Hypertens* 2004 January;17(1):31-6.

(828) Ketonen J, Merasto S, Paakkari I, Mervaala EM. High sodium intake increases vascular superoxide formation and promotes atherosclerosis in apolipoprotein E-deficient mice. *Blood Press* 2005;14(6):373-82.

(829) Lenda DM, Boegehold MA. Effect of a high-salt diet on oxidant enzyme activity in skeletal muscle microcirculation. *Am J Physiol Heart Circ Physiol* 2002 February;282(2):H395-H402.

(830) Wagner AH, Kohler T, Ruckschloss U, Just I, Hecker M. Improvement of nitric oxide-dependent vasodilatation by HMG-CoA reductase inhibitors through attenuation of endothelial superoxide anion formation. *Arterioscler Thromb Vasc Biol* 2000 January;20(1):61-9.

(831) Vecchione C, Brandes RP. Withdrawal of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors elicits oxidative stress and induces endothelial dysfunction in mice. *Circ Res* 2002 July 26;91(2):173-9.

(832) Endres M, Laufs U. Effects of statins on endothelium and signaling mechanisms. *Stroke* 2004 November;35(11 Suppl 1):2708-11.

(833) Rueckschloss U, Quinn MT, Holtz J, Morawietz H. Dose-dependent regulation of NAD(P)H oxidase expression by angiotensin II in human endothelial cells: protective effect of angiotensin II type 1 receptor blockade in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol* 2002 November 1;22(11):1845-51.

(834) Desideri G, Bravi MC, Tucci M et al. Angiotensin II inhibits endothelial cell motility through an AT1-dependent oxidant-sensitive decrement of nitric oxide availability. *Arterioscler Thromb Vasc Biol* 2003 July 1;23(7):1218-23.

(835) Girouard H, Park L, Anrather J, Zhou P, Iadecola C. Angiotensin II attenuates endothelium-dependent responses in the cerebral microcirculation through nox-2-derived radicals. *Arterioscler Thromb Vasc Biol* 2006 April;26(4):826-32.

(836) Gilca M, Stoian I, Atanasiu V, Virgolici B. The oxidative hypothesis of senescence. *J Postgrad Med* 2007 July;53(3):207-13.

(837) Ku HH, Brunk UT, Sohal RS. Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species. *Free Radic Biol Med* 1993 December;15(6):621-7.

(838) Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996 July 5;273(5271):59-63.

(839) Bartke A. Minireview: role of the growth hormone/insulin-like growth factor system in mammalian aging. *Endocrinology* 2005 September;146(9):3718-23.

(840) Bartke A. Impact of reduced insulin-like growth factor-1/insulin signaling on aging in mammals: novel findings. *Aging Cell* 2008 June;7(3):285-90.

(841) Schriner SE, Linford NJ, Martin GM et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 2005 June 24;308(5730):1909-11.

(842) Trowell HC. *Non-Infectious Disease in Africa*. London: Edward Arnold; 1960.

(843) Trowell HC, Burkitt DP. *Western Diseases: Their Emergence and Prevention*. Cambridge, MA: Harvard University Press; 1981.

(844) Lindeberg S, Lundh B. Apparent absence of stroke and ischaemic heart disease in a traditional Melanesian island: a clinical study in Kitava. *J Intern Med* 1993 March;233(3):269-75.

(845) McCarty MF. Up-regulation of endothelial nitric oxide activity as a central strategy for prevention of ischemic stroke - just say NO to stroke! *Med Hypotheses* 2000 November;55(5):386-403.

(846) Bayard V, Chamorro F, Motta J, Hollenberg NK. Does flavanol intake influence mortality from nitric oxide-dependent processes? Ischemic heart disease, stroke, diabetes mellitus, and cancer in Panama. *Int J Med Sci* 2007;4(1):53-8.

(847) McCarty MF. Insulin and IGF-I as determinants of low "Western" cancer rates in the rural third world. *Int J Epidemiol* 2004 August;33(4):908-10.

(848) McCarty MF. Point of view--a role for "nutritional insurance" supplementation in preventive medicine. *Med Hypotheses* 1981 February;7(2):171-85.

(849) Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? *Br J Nutr* 2003 May;89(5):552-72.

(850) Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004 December;80(6 Suppl):1678S-88S.

(851) Vieth R. Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. *J Steroid Biochem Mol Biol* 2004 May;89-90(1-5):575-9.

(852) Schwartz GG, Whitlatch LW, Chen TC, Lokeshwar BL, Holick MF. Human prostate cells synthesize 1,25-dihydroxyvitamin D3 from 25-hydroxyvitamin D3. *Cancer Epidemiol Biomarkers Prev* 1998 May;7(5):391-5.

(853) Townsend K, Evans KN, Campbell MJ, Colston KW, Adams JS, Hewison M. Biological actions of extra-renal 25-hydroxyvitamin D-1alpha-hydroxylase and implications for chemoprevention and treatment. *J Steroid Biochem Mol Biol* 2005 October;97(1-2):103-9.

(854) Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. *Cancer* 2002 March 15;94(6):1867-75.

(855) Grant WB. The likely role of vitamin D from solar ultraviolet-B irradiance in increasing cancer survival. *Anticancer Res* 2006 July;26(4A):2605-14.

(856) Gorham ED, Garland CF, Garland FC et al. Vitamin D and prevention of colorectal cancer. *J Steroid Biochem Mol Biol* 2005 October;97(1-2):179-94.

(857) Gorham ED, Garland CF, Garland FC et al. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. *Am J Prev Med* 2007 March;32(3):210-6.

(858) Grant WB, Garland CF, Gorham ED. An estimate of cancer mortality rate reductions in Europe and the US with 1,000 IU of oral vitamin D per day. *Recent Results Cancer Res* 2007;174:225-34.

(859) Ainsleigh HG. Beneficial effects of sun exposure on cancer mortality. *Prev Med* 1993 January;22(1):132-40.

(860) Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int J Epidemiol* 1980 September;9(3):227-31.

(861) Stoffels K, Overbergh L, Bouillon R, Mathieu C. Immune regulation of 1alpha-hydroxylase in murine peritoneal macrophages: unravelling the IFNgamma pathway. *J Steroid Biochem Mol Biol* 2007 March;103(3-5):567-71.

(862) Adorini L, Penna G. Control of autoimmune diseases by the vitamin D endocrine system. *Nat Clin Pract Rheumatol* 2008 August;4(8):404-12.

(863) van EE, Decallonne B, Mathieu C. 1,25-dihydroxycholecalciferol: endocrinology meets the immune system. *Proc Nutr Soc* 2002 August;61(3):375-80.

(864) Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *J Immunol* 2007 August 15;179(4):2060-3.

(865) Hata TR, Kotol P, Jackson M et al. Administration of oral vitamin D induces cathelicidin production in atopic individuals. *J Allergy Clin Immunol* 2008 October;122(4):829-31.

(866) McCarty MF. Nutritional modulation of parathyroid hormone secretion may influence risk for left ventricular hypertrophy. *Med Hypotheses* 2005;64(5):1015-21.

(867) Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). *Am J Cardiol* 2008 December 1;102(11):1540-4.

(868) Zittermann A, Koerfer R. Vitamin D in the prevention and treatment of coronary heart disease. *Curr Opin Clin Nutr Metab Care* 2008 November;11(6):752-7.

(869) Michos ED, Melamed ML. Vitamin D and cardiovascular disease risk. *Curr Opin Clin Nutr Metab Care* 2008 January;11(1):7-12.

(870) Zittermann A, Schleithoff SS, Koerfer R. Vitamin D insufficiency in congestive heart failure: why and what to do about it? *Heart Fail Rev* 2006 March;11(1):25-33.

(871) Pilz S, Dobnig H, Fischer JE et al. Low vitamin d levels predict stroke in patients referred to coronary angiography. *Stroke* 2008 September;39(9):2611-3.

(872) Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* 2008 June 9;168(11):1174-80.

(873) Knekt P, Laaksonen M, Mattila C et al. Serum vitamin D and subsequent occurrence of type 2 diabetes. *Epidemiology* 2008 September;19(5):666-71.

(874) Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. *Hypertension* 2008 November;52(5):828-32.

(875) Martini LA, Wood RJ. Vitamin D and blood pressure connection: update on epidemiologic, clinical, and mechanistic evidence. *Nutr Rev* 2008 May;66(5):291-7.

(876) Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. *Am J Clin Nutr* 2001 February;73(2):288-94.

(877) Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999 May;69(5):842-56.

(878) Vieth R. Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. *J Steroid Biochem Mol Biol* 2004 May;89-90(1-5):575-9.

(879) Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. *Bone* 1996 May;18(5):487-8.

(880) Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. *J Clin Endocrinol Metab* 1997 March;82(3):719-24.

(881) Binkley NC, Krueger DC, Kawahara TN, Engelke JA, Chappell RJ, Suttie JW. A high phylloquinone intake is required to achieve maximal osteocalcin gamma-carboxylation. *Am J Clin Nutr* 2002 November;76(5):1055-60.

(882) Francucci CM, Rilli S, Fiscaletti P, Boscaro M. Role of vitamin K on biochemical markers, bone mineral density, and fracture risk. *J Endocrinol Invest* 2007;30(6 Suppl):24-8.

(883) Bugel S. Vitamin K and bone health in adult humans. *Vitam Horm* 2008;78:393-416.

(884) Cockayne S, Adamson J, Lanham-New S, Shearer MJ, Gilbody S, Torgerson DJ. Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. *Arch Intern Med* 2006 June 26;166(12):1256-61.

(885) Iwamoto J, Takeda T, Sato Y. Role of vitamin K2 in the treatment of postmenopausal osteoporosis. *Curr Drug Saf* 2006 January;1(1):87-97.

(886) Kaneki M, Hodges SJ, Hosoi T et al. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. *Nutrition* 2001 April;17(4):315-21.

(887) Katsuyama H, Ideguchi S, Fukunaga M, Saijoh K, Sunami S. Usual dietary intake of fermented soybeans (Natto) is associated with bone mineral density in premenopausal women. *J Nutr Sci Vitaminol (Tokyo)* 2002 June;48(3):207-15.

(888) Kaneki M, Hosoi T, Ouchi Y, Orimo H. Pleiotropic actions of vitamin K: protector of bone health and beyond? *Nutrition* 2006 July;22(7-8):845-52.

(889) Shanahan CM, Proudfoot D, Farzaneh-Far A, Weissberg PL. The role of Gla proteins in vascular calcification. *Crit Rev Eukaryot Gene Expr* 1998;8(3-4):357-75.

(890) Erkkila AT, Booth SL. Vitamin K intake and atherosclerosis. *Curr Opin Lipidol* 2008 February;19(1):39-42.

(891) Geleijnse JM, Vermeer C, Grobbee DE et al. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr* 2004 November;134(11):3100-5.

(892) Beulens JW, Bots ML, Atsma F et al. High dietary menaquinone intake is associated with reduced coronary calcification. *Atherosclerosis* 2008 July 19.

(893) Braam LA, Hoeks AP, Brouns F, Hamulyak K, Gerichhausen MJ, Vermeer C. Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in

postmenopausal women: a follow-up study. *Thromb Haemost* 2004 February;91(2):373-80.

(894) Suzuki M, Shiraha H, Fujikawa T et al. Des-gamma-carboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. *J Biol Chem* 2005 February 25;280(8):6409-15.

(895) Carr BI, Kar S, Wang M, Wang Z. Growth inhibitory actions of prothrombin on normal hepatocytes: influence of matrix. *Cell Biol Int* 2007 September;31(9):929-38.

(896) Bertino G, Ardiri AM, Boemi PM et al. A study about mechanisms of des-gamma-carboxy prothrombin's production in hepatocellular carcinoma. *Panminerva Med* 2008 September;50(3):221-6.

(897) Ma M, Qu XJ, Mu GY et al. Vitamin K(2) Inhibits the Growth of Hepatocellular Carcinoma via Decrease of Des-Gamma-Carboxy Prothrombin. *Chemotherapy* 2008 October 31;55(1):28-35.

(898) Hitomi M, Nonomura T, Yokoyama F et al. In vitro and in vivo antitumor effects of vitamin K5 on hepatocellular carcinoma. *Int J Oncol* 2005 May;26(5):1337-44.

(899) Habu D, Shiomi S, Tamori A et al. Role of vitamin K2 in the development of hepatocellular carcinoma in women with viral cirrhosis of the liver. *JAMA* 2004 July 21;292(3):358-61.

(900) Kakizaki S, Sohara N, Sato K et al. Preventive effects of vitamin K on recurrent disease in patients with hepatocellular carcinoma arising from hepatitis C viral infection. *J Gastroenterol Hepatol* 2007 April;22(4):518-22.

(901) Mizuta T, Ozaki I, Eguchi Y et al. The effect of menatetrenone, a vitamin K2 analog, on disease recurrence and survival in patients with hepatocellular carcinoma after curative treatment: a pilot study. *Cancer* 2006 February 15;106(4):867-72.

(902) Tamori A, Habu D, Shiomi S, Kubo S, Nishiguchi S. Potential role of vitamin K(2) as a chemopreventive agent against hepatocellular carcinoma. *Hepatol Res* 2007 September;37 Suppl 2:S303-S307.

(903) Nouso K, Uematsu S, Shiraga K et al. Regression of hepatocellular carcinoma during vitamin K administration. *World J Gastroenterol* 2005 November 14;11(42):6722-4.

(904) Sarin SK, Kumar M, Garg S, Hissar S, Pandey C, Sharma BC. High dose vitamin K3 infusion in advanced hepatocellular carcinoma. *J Gastroenterol Hepatol* 2006 September;21(9):1478-82.

(905) Honkanen R, Karkkainen M, Kroger H. Calcium and vitamin D in promotion of postmenopausal bone health. *Womens Health (Lond Engl)* 2010 November;6(6):773-6.

(906) Patient level pooled analysis of 68 500 patients from seven major vitamin D fracture trials in US and Europe. *BMJ* 2010;340:b5463.

(907) Strause L, Saltman P, Smith KT, Bracker M, Andon MB. Spinal bone loss in postmenopausal women supplemented with calcium and trace minerals. *J Nutr* 1994 July;124(7):1060-4.

(908) Carroll C, Cooper K, Papaioannou D, Hind D, Pilgrim H, Tappenden P. Supplemental calcium in the chemoprevention of colorectal cancer: a systematic review and meta-analysis. *Clin Ther* 2010 May;32(5):789-803.

(909) Bolland MJ, Avenell A, Baron JA et al. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ* 2010;341:c3691.

(910) Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ* 2011;342:d2040.

(911) Nordin BE, Lewis JR, Daly RM et al. The calcium scare-what would Austin Bradford Hill have thought? *Osteoporos Int* 2011 June 2.

(912) Seelig MS. Interrelationship of magnesium and estrogen in cardiovascular and bone disorders, eclampsia, migraine and premenstrual syndrome. *J Am Coll Nutr* 1993 August;12(4):442-58.

(913) Lijnen P, Petrov V. Effect of oral calcium supplementation on intracellular calcium and plasma renin in men. *J Endocrinol* 1995 September;146(3):421-9.

(914) McCarty MF. Calcium supplementation, renin, and vascular risk. *Osteoporos Int* 2012 November;23(11):2733-4.

(915) Seelig MS, Heggtveit HA. Magnesium interrelationships in ischemic heart disease: a review. *Am J Clin Nutr* 1974 January;27(1):59-79.

(916) Ueshima K. Magnesium and ischemic heart disease: a review of epidemiological, experimental, and clinical evidences. *Magnes Res* 2005 December;18(4):275-84.

(917) Guerrero-Romero F, Rodriguez-Moran M. The effect of lowering blood pressure by magnesium supplementation in diabetic hypertensive adults with low serum magnesium levels: a randomized, double-blind, placebo-controlled clinical trial. *J Hum Hypertens* 2008 November 20.

(918) Shechter M, Merz CN, Paul-Labrador M et al. Oral magnesium supplementation inhibits platelet-dependent thrombosis in patients with coronary artery disease. *Am J Cardiol* 1999 July 15;84(2):152-6.

(919) Shechter M, Sharir M, Labrador MJ, Forrester J, Silver B, Bairey Merz CN. Oral magnesium therapy improves endothelial function in patients with coronary artery disease. *Circulation* 2000 November 7;102(19):2353-8.

(920) Shechter M, Bairey Merz CN, Stuehlinger HG, Slany J, Pachinger O, Rabinowitz B. Effects of oral magnesium therapy on exercise tolerance, exercise-induced chest pain, and quality of life in patients with coronary artery disease. *Am J Cardiol* 2003 March 1;91(5):517-21.

(921) Shechter M. Does magnesium have a role in the treatment of patients with coronary artery disease? *Am J Cardiovasc Drugs* 2003;3(4):231-9.

(922) Feiner JJ, McNurlan MA, Ferris RE, Mynarcik DC, Gelato MC. Chromium picolinate for insulin resistance in subjects with HIV disease: a pilot study. *Diabetes Obes Metab* 2008 February;10(2):151-8.

(923) Martin J, Wang ZQ, Zhang XH et al. Chromium picolinate supplementation attenuates body weight gain and increases insulin sensitivity in subjects with type 2 diabetes. *Diabetes Care* 2006 August;29(8):1826-32.

(924) Lydic ML, McNurlan M, Bembo S, Mitchell L, Komaroff E, Gelato M. Chromium picolinate improves insulin sensitivity in obese subjects with polycystic ovary syndrome. *Fertil Steril* 2006 July;86(1):243-6.

(925) Wang ZQ, Qin J, Martin J et al. Phenotype of subjects with type 2 diabetes mellitus may determine clinical response to chromium supplementation. *Metabolism* 2007 December;56(12):1652-5.

(926) Anderson RA, Cheng N, Bryden NA et al. Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes* 1997 November;46(11):1786-91.

(927) Anton SD, Morrison CD, Cefalu WT et al. Effects of chromium picolinate on food intake and satiety. *Diabetes Technol Ther* 2008 October;10(5):405-12.

(928) Jugdaohsingh R, Tucker KL, Qiao N, Cupples LA, Kiel DP, Powell JJ. Dietary silicon intake is positively associated with bone mineral density in men and premenopausal women of the Framingham Offspring cohort. *J Bone Miner Res* 2004 February;19(2):297-307.

(929) Calomme M, Geusens P, Demeester N et al. Partial prevention of long-term femoral bone loss in aged ovariectomized rats supplemented with choline-stabilized orthosilicic acid. *Calcif Tissue Int* 2006 April;78(4):227-32.

(930) Jugdaohsingh R. Silicon and bone health. *J Nutr Health Aging* 2007 March;11(2):99-110.

(931) Schwarz K. Silicon, fibre, and atherosclerosis. *Lancet* 1977 February 26;1(8009):454-7.

(932) Loeper J, Goy-Loeper J, Rozensztajn L, Fragny M. The antiatheromatous action of silicon. *Atherosclerosis* 1979 August;33(4):397-408.

(933) Newnham RE. Essentiality of boron for healthy bones and joints. *Environ Health Perspect* 1994 November;102 Suppl 7:83-5.

(934) Nielsen FH. Is boron nutritionally relevant? *Nutr Rev* 2008 April;66(4):183-91.

(935) Ammann P. Strontium ranelate: a physiological approach for an improved bone quality. *Bone* 2006 February;38(2 Suppl 1):15-8.

(936) Tournis S. Improvement in bone strength parameters. The role of strontium ranelate. *J Musculoskelet Neuronal Interact* 2007 July;7(3):266-7.

(937) Blake GM, Fogelman I. Strontium ranelate: a novel treatment for postmenopausal osteoporosis: a review of safety and efficacy. *Clin Interv Aging* 2006;1(4):367-75.

(938) Seeman E. Strontium ranelate: vertebral and non-vertebral fracture risk reduction. *Curr Opin Rheumatol* 2006 June;18 Suppl 1:S17-S20.

(939) O'Donnell S, Cranney A, Wells GA, Adachi JD, Reginster JY. Strontium ranelate for preventing and treating postmenopausal osteoporosis. *Cochrane Database Syst Rev* 2006;(4):CD005326.

(940) Meunier PJ, Slosman DO, Delmas PD et al. Strontium ranelate: dose-dependent effects in established postmenopausal vertebral osteoporosis--a 2-year randomized placebo controlled trial. *J Clin Endocrinol Metab* 2002 May;87(5):2060-6.

(941) Curzon ME. The relation between caries prevalence and strontium concentrations in drinking water, plaque, and surface enamel. *J Dent Res* 1985 December;64(12):1386-8.

(942) Riyat M, Sharma DC. Analysis of 35 inorganic elements in teeth in relation to caries formation. *Biol Trace Elem Res* 2009;129(1-3):126-9.

(943) Henrotin Y, Labasse A, Zheng SX et al. Strontium ranelate increases cartilage matrix formation. *J Bone Miner Res* 2001 February;16(2):299-308.

(944) Alexandersen P, Karsdal MA, Qvist P, Reginster JY, Christiansen C. Strontium ranelate reduces the urinary level of cartilage degradation biomarker CTX-II in postmenopausal women. *Bone* 2007 January;40(1):218-22.

(945) Bruyere O, Delferriere D, Roux C et al. Effects of strontium ranelate on spinal osteoarthritis progression. *Ann Rheum Dis* 2008 March;67(3):335-9.

(946) Sublette ME, Hibbeln JR, Galfalvy H, Oquendo MA, Mann JJ. Omega-3 polyunsaturated essential fatty acid status as a predictor of future suicide risk. *Am J Psychiatry* 2006 June;163(6):1100-2.

(947) McNamara RK, Carlson SE. Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot Essent Fatty Acids* 2006 October;75(4-5):329-49.

(948) Hibbeln JR. From homicide to happiness--a commentary on omega-3 fatty acids in human society. Cleave Award Lecture. *Nutr Health* 2007;19(1-2):9-19.

(949) Rotstein NP, Politi LE, German OL, Girotti R. Protective effect of docosahexaenoic acid on oxidative stress-induced apoptosis of retina photoreceptors. *Invest Ophthalmol Vis Sci* 2003 May;44(5):2252-9.

(950) Mitchell DC, Niu SL, Litman BJ. DHA-rich phospholipids optimize G-Protein-coupled signaling. *J Pediatr* 2003 October;143(4 Suppl):S80-S86.

(951) Rotstein NP, Aveldano MI, Barrantes FJ, Roccamo AM, Politi LE. Apoptosis of retinal photoreceptors during development in vitro: protective effect of docosahexaenoic acid. *J Neurochem* 1997 August;69(2):504-13.

(952) Mukherjee PK, Marcheselli VL, Barreiro S, Hu J, Bok D, Bazan NG. Neurotrophins enhance retinal pigment epithelial cell survival through neuroprotectin D1 signaling. *Proc Natl Acad Sci U S A* 2007 August 7;104(32):13152-7.

(953) Chong EW, Kreis AJ, Wong TY, Simpson JA, Guymer RH. Dietary omega-3 fatty acid and fish intake in the primary prevention of age-related macular degeneration: a systematic review and meta-analysis. *Arch Ophthalmol* 2008 June;126(6):826-33.

(954) Sangiovanni JP, Agron E, Meleth AD et al. {omega}-3 Long-chain polyunsaturated fatty acid intake and 12-y incidence of neovascular age-related macular degeneration and central geographic atrophy: AREDS report 30, a prospective cohort study from the Age-Related Eye Disease Study. *Am J Clin Nutr* 2009 December;90(6):1601-7.

(955) Leaf A. Prevention of sudden cardiac death by n-3 polyunsaturated fatty acids. *J Cardiovasc Med (Hagerstown)* 2007 September;8 Suppl 1:S27-S29.

(956) Chung MK. Omega-3 Fatty Acid supplementation for the prevention of arrhythmias. *Curr Treat Options Cardiovasc Med* 2008 September;10(5):398-407.

(957) Zampelas A, Panagiotakos DB, Pitsavos C et al. Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease: the ATTICA study. *J Am Coll Cardiol* 2005 July 5;46(1):120-4.

(958) van Bussel BC, Henry RM, Schalkwijk CG et al. Fish consumption in healthy adults is associated with decreased circulating biomarkers of endothelial dysfunction and inflammation during a 6-year follow-up. *J Nutr* 2011 September;141(9):1719-25.

(959) Micallef MA, Munro IA, Garg ML. An inverse relationship between plasma n-3 fatty acids and C-reactive protein in healthy individuals. *Eur J Clin Nutr* 2009 September;63(9):1154-6.

(960) Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999 August 7;354(9177):447-55.

(961) Kremer JM, Jubiz W, Michalek A et al. Fish-oil fatty acid supplementation in active rheumatoid arthritis. A double-blinded, controlled, crossover study. *Ann Intern Med* 1987 April;106(4):497-503.

(962) Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* 2006 June;83(6 Suppl):1505S-19S.

(963) Dyerberg J, Bang HO. Lipid metabolism, atherogenesis, and haemostasis in Eskimos: the role of the prostaglandin-3 family. *Haemostasis* 1979;8(3-5):227-33.

(964) Hay CR, Durber AP, Saynor R. Effect of fish oil on platelet kinetics in patients with ischaemic heart disease. *Lancet* 1982 June 5;1(8284):1269-70.

(965) Goodnight SH, Jr., Harris WS, Connor WE. The effects of dietary omega 3 fatty acids on platelet composition and function in man: a prospective, controlled study. *Blood* 1981 November;58(5):880-5.

(966) Howe PR. Can we recommend fish oil for hypertension? *Clin Exp Pharmacol Physiol* 1995 March;22(3):199-203.

(967) Yang H, Kenny A. The role of fish oil in hypertension. *Conn Med* 2007 October;71(9):533-8.

(968) Deckelbaum RJ, Torrejon C. The omega-3 fatty acid nutritional landscape: health benefits and sources. *J Nutr* 2012 March;142(3):587S-91S.

(969) Walker CG, Jebb SA, Calder PC. Stearidonic acid as a supplemental source of omega-3 polyunsaturated fatty acids to enhance status for improved human health. *Nutrition* 2013 February;29(2):363-9.

(970) Lemke SL, Vicini JL, Su H et al. Dietary intake of stearidonic acid-enriched soybean oil increases the omega-3 index: randomized, double-blind clinical study of efficacy and safety. *Am J Clin Nutr* 2010 October;92(4):766-75.

(971) Kuiper GG, Lemmen JG, Carlsson B et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 1998 October;139(10):4252-63.

(972) McCarty MF. Isoflavones made simple - genistein's agonist activity for the beta-type estrogen receptor mediates their health benefits. *Med Hypotheses* 2006;66(6):1093-114.

(973) Yan L, Spitznagel EL. Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. *Am J Clin Nutr* 2009 April;89(4):1155-63.

(974) Wu AH, Yu MC, Tseng CC, Pike MC. Epidemiology of soy exposures and breast cancer risk. *Br J Cancer* 2008 January 15;98(1):9-14.

(975) Yang G, Shu XO, Li H et al. Prospective cohort study of soy food intake and colorectal cancer risk in women. *Am J Clin Nutr* 2009 February;89(2):577-83.

(976) Jackson RL, Greiwe JS, Schwen RJ. Emerging evidence of the health benefits of S-equol, an estrogen receptor beta agonist. *Nutr Rev* 2011 August;69(8):432-48.

(977) Weaver CM, Legette LL. Equol, via dietary sources or intestinal production, may ameliorate estrogen deficiency-induced bone loss. *J Nutr* 2010 July;140(7):1377S-9S.

(978) Guha N, Kwan ML, Quesenberry CP, Jr., Weltzien EK, Castillo AL, Caan BJ. Soy isoflavones and risk of cancer recurrence in a cohort of breast cancer survivors: the Life After Cancer Epidemiology study. *Breast Cancer Res Treat* 2009 November;118(2):395-405.

(979) Caan BJ, Natarajan L, Parker B et al. Soy food consumption and breast cancer prognosis. *Cancer Epidemiol Biomarkers Prev* 2011 May;20(5):854-8.

(980) Kang HB, Zhang YF, Yang JD, Lu KL. Study on soy isoflavone consumption and risk of breast cancer and survival. *Asian Pac J Cancer Prev* 2012;13(3):995-8.

(981) Zhang YF, Kang HB, Li BL, Zhang RM. Positive effects of soy isoflavone food on survival of breast cancer patients in China. *Asian Pac J Cancer Prev* 2012;13(2):479-82.

(982) van Dam RM, Dekker JM, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ. Coffee consumption and incidence of impaired fasting glucose, impaired glucose tolerance, and type 2 diabetes: the Hoorn Study. *Diabetologia* 2004 December;47(12):2152-9.

(983) van Dam RM, Willett WC, Manson JE, Hu FB. Coffee, caffeine, and risk of type 2 diabetes: a prospective cohort study in younger and middle-aged U.S. women. *Diabetes Care* 2006 February;29(2):398-403.

(984) van Dam RM. Coffee and type 2 diabetes: from beans to beta-cells. *Nutr Metab Cardiovasc Dis* 2006 January;16(1):69-77.

(985) Johnston KL, Clifford MN, Morgan LM. Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. *Am J Clin Nutr* 2003 October;78(4):728-33.

(986) McCarty MF. A chlorogenic acid-induced increase in GLP-1 production may mediate the impact of heavy coffee consumption on diabetes risk. *Med Hypotheses* 2005;64(4):848-53.

(987) Vinson JA, Burnham BR, Nagendran MV. Randomized, double-blind, placebo-controlled, linear dose, crossover study to evaluate the efficacy and safety of a green coffee bean extract in overweight subjects. *Diabetes Metab Syndr Obes* 2012;5:21-7.

(988) McCarty MF. Enhanced synovial production of hyaluronic acid may explain rapid clinical response to high-dose glucosamine in osteoarthritis. *Med Hypotheses* 1998 June;50(6):507-10.

(989) Uitterlinden EJ, Koevoet JL, Verkoelen CF et al. Glucosamine increases hyaluronic acid production in human osteoarthritic synovium explants. *BMC Musculoskelet Disord* 2008;9:120.

(990) McCarty MF. The neglect of glucosamine as a treatment for osteoarthritis--a personal perspective. *Med Hypotheses* 1994 May;42(5):323-7.

(991) Zerkak D, Dougados M. The use of glucosamine therapy in osteoarthritis. *Curr Rheumatol Rep* 2004 February;6(1):41-5.

(992) Towheed TE, Maxwell L, Anastassiades TP et al. Glucosamine therapy for treating osteoarthritis. *Cochrane Database Syst Rev* 2005;(2):CD002946.

(993) Poolsup N, Suthisisang C, Channark P, Kittikulsuth W. Glucosamine long-term treatment and the progression of knee osteoarthritis: systematic review of randomized controlled trials. *Ann Pharmacother* 2005 June;39(6):1080-7.

(994) Reginster JY, Bruyere O, Neuprez A. Current role of glucosamine in the treatment of osteoarthritis. *Rheumatology (Oxford)* 2007 May;46(5):731-5.

(995) Reginster JY, Deroisy R, Rovati LC et al. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. *Lancet* 2001 January 27;357(9252):251-6.

(996) Pavelka K, Gatterova J, Olejarova M, Machacek S, Giacovelli G, Rovati LC. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. *Arch Intern Med* 2002 October 14;162(18):2113-23.

(997) Bruyere O, Pavelka K, Rovati LC et al. Glucosamine sulfate reduces osteoarthritis progression in postmenopausal women with knee osteoarthritis: evidence from two 3-year studies. *Menopause* 2004 March;11(2):138-43.

(998) Bruyere O, Pavelka K, Rovati LC et al. Total joint replacement after glucosamine sulphate treatment in knee osteoarthritis: results of a mean 8-year observation of patients from two previous 3-year, randomised, placebo-controlled trials. *Osteoarthritis Cartilage* 2008 February;16(2):254-60.

(999) Neil KM, Orth MW, Coussens PM, Chan PS, Caron JP. Effects of glucosamine and chondroitin sulfate on mediators of osteoarthritis in cultured equine chondrocytes stimulated by use of recombinant equine interleukin-1beta. *Am J Vet Res* 2005 November;66(11):1861-9.

(1000) Gouze JN, Gouze E, Popp MP et al. Exogenous glucosamine globally protects chondrocytes from the arthritogenic effects of IL-1beta. *Arthritis Res Ther* 2006;8(6):R173.

(1001) Jang BC, Sung SH, Park JG et al. Glucosamine hydrochloride specifically inhibits COX-2 by preventing COX-2 N-glycosylation and by increasing COX-2 protein turnover in a proteasome-dependent manner. *J Biol Chem* 2007 September 21;282(38):27622-32.

(1002) Valvason C, Musacchio E, Pozzuoli A, Ramonda R, Aldegheri R, Punzi L. Influence of glucosamine sulphate on oxidative stress in human osteoarthritic chondrocytes: effects on HO-1, p22(Phox) and iNOS expression. *Rheumatology (Oxford)* 2008 January;47(1):31-5.

(1003) Lu HT, Liang YC, Sheu MT et al. Disease-modifying effects of glucosamine HCl involving regulation of metalloproteinases and chemokines activated by interleukin-1beta in human primary synovial fibroblasts. *J Cell Biochem* 2008 May 1;104(1):38-50.

(1004) Clegg DO, Reda DJ, Harris CL et al. Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis. *N Engl J Med* 2006 February 23;354(8):795-808.

(1005) Tiraloche G, Girard C, Chouinard L et al. Effect of oral glucosamine on cartilage degradation in a rabbit model of osteoarthritis. *Arthritis Rheum* 2005 April;52(4):1118-28.

(1006) Wang SX, Laverty S, Dumitriu M, Plaas A, Grynpas MD. The effects of glucosamine hydrochloride on subchondral bone changes in an animal model of osteoarthritis. *Arthritis Rheum* 2007 May;56(5):1537-48.

(1007) Virkamaki A, Daniels MC, Hamalainen S, Utriainen T, McClain D, Yki-Jarvinen H. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance in multiple insulin sensitive tissues. *Endocrinology* 1997 June;138(6):2501-7.

(1008) Baron AD, Zhu JS, Zhu JH, Weldon H, Maianu L, Garvey WT. Glucosamine induces insulin resistance in vivo by affecting GLUT 4 translocation in skeletal muscle. Implications for glucose toxicity. *J Clin Invest* 1995 December;96(6):2792-801.

(1009) Scroggie DA, Albright A, Harris MD. The effect of glucosamine-chondroitin supplementation on glycosylated hemoglobin levels in patients with type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. *Arch Intern Med* 2003 July 14;163(13):1587-90.

(1010) Anderson JW, Nicolosi RJ, Borzelleca JF. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. *Food Chem Toxicol* 2005 February;43(2):187-201.

(1011) Stumpf JL, Lin SW. Effect of glucosamine on glucose control. *Ann Pharmacother* 2006 April;40(4):694-8.

(1012) Biggee BA, Blinn CM, Nuite M, Silbert JE, McAlindon TE. Effects of oral glucosamine sulphate on serum glucose and insulin during an oral glucose tolerance test of subjects with osteoarthritis. *Ann Rheum Dis* 2007 February;66(2):260-2.

(1013) Muniyappa R, Karne RJ, Hall G et al. Oral glucosamine for 6 weeks at standard doses does not cause or worsen insulin resistance or endothelial dysfunction in lean or obese subjects. *Diabetes* 2006 November;55(11):3142-50.

(1014) Pocobelli G, Kristal AR, Patterson RE et al. Total mortality risk in relation to use of less-common dietary supplements. *Am J Clin Nutr* 2010 June;91(6):1791-800.

(1015) Bell GA, Kantor ED, Lampe JW, Shen DD, White E. Use of glucosamine and chondroitin in relation to mortality. *Eur J Epidemiol* 2012 August;27(8):593-603.

(1016) Satia JA, Littman A, Slatore CG, Galanko JA, White E. Associations of herbal and specialty supplements with lung and colorectal cancer risk in the VITamins and Lifestyle study. *Cancer Epidemiol Biomarkers Prev* 2009 May;18(5):1419-28.