# Full-Spectrum Antioxidant Therapy

## Minimizing the Contribution of Oxidative Stress to Disease and Aging

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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.

I should emphasize that I am not proposing 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, I am using the term "Therapy" 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 (and don't let the molecular biology buffalo you – we'll soon explain it):

- **Partial suppression of NADPH oxidase activity** by ingestion of spirulina or phycocyanobilin-enriched spirulina extracts;
- **Scavenging of peroxynitrite-derived radicals** by supplementation with **high-dose folate** and, optionally, **inosine** or or dietary nucleic acids;
- **Protecting mitochondrial structure and function** with supplemental **Coenzyme Q10**;
- Induction of antioxidant enzymes and promotion of glutathione synthesis with **phase 2-inducing nutraceuticals –** most notably **alpha-lipoic acid** - 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**.

Now let's try to explain what that means!

## 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 to your body's tissues; 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.

But the most intriguing factor generated by HO-1 activity is bilirubin. Bilirubin is extremely insoluble; the liver conjugates it (that is, links it to a sugar molecule) so that is becomes soluble enough 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. 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.

To understand the answer, you first need to know something about where free radicals come from. 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, but in excess it can lead to cell death or dysfunction, in part because it can interact with free iron 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.

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. (In fact, this complex occurs in several different forms, but 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 acts 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[2] 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.[3]

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 reflects its ability to act as a very potent inhibitor of NAPDH oxidase activity***.[4-6]

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.[7] 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 to 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).[2, 8] 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.[9] 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 sugar molecule (that is, conjugates it) 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 have this "problem" are said to have Gilbert syndrome (in honor of the French physician who first characterized it). 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.[10] 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.[11] (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!)[12]

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.

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 partially inhibits UGT1A1 activity in the liver – a strategy that has been dubbed "iatrogenic Gilbert syndrome".[2] ("Iatrogenic" means "physician-induced".) There are indeed some drugs which have this activity, and clinical work exploring this approach will likely be carried out in the future. 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,[13] 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 is 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 tightly 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! Take a look at the two structures; it may take you awhile to spot the differences.

[Figure 1]

In fact the only differences occur at the far ends of the molecules. 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 bilverdin 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. Well, 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.[14] Take a look at the comparative structures of bilirubin and phycocyanorubin:

[Figure 2]

Not easy to tell the difference, is it?

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. Here's what he found:

[Figure 3]

As you can see, the PhyCB worked virtually as well as biliverdin in quelling oxidative stress in these 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 or India – demonstrating that oral ingestion of phycocyanin exerts a very broad range of anti-inflammatory, antioxidant, and cytoprotective effects.[15, 16] 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 give you an idea of the scope of this research, take a look at a table from a review article that summarizes the rodent studies that Cuban scientists have conducted with oral phycocyanin.[15] It cites 12 different models in which phycocyanin has exerted anti-inflammatory or cell-protective effects. And note that 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.[17] 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,[18, 19] including Alzheimer's, Parkinson's, and ALS ("Lou Gehrig's disease"), the ability of PhyCB to penetrate the brain may have exciting implications for the prevention and possibly even control of these tragic afflictions.

[Table 1]

Until recently, no would have 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%.[20] Even more recently, Dr. Inoguchi has observed that, in genetically obese diabetic mice, feeding either biliverdin or phycocyanin largely prevents diabetic kidney damage – decreasing the urinary loss of albumin, and almost eliminating oxidative stress and the sclerotic response in the filtering units of the kidneys (glomeruli). (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.

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.[16]

But 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. 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[21, 22] – in particular, this doesn't harm the reproductive process[23, 24] (good news in light of the fact that overactivity of NADPH oxidase is a mediator of the common pregnancy syndrome pre-eclampsia).[25] 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.

## Coping with Peroxynitrite

As you may recall, one of the key mediators of the tissue damage induced by oxidative stress is a compound known as peroxynitrite. This compound 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.[26] 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, liver, or bladder. When peroxynitrite-mediated DNA damage in a cell is extensive, this causes overactivation of an enzyme called PARP (we'll spare you the full name!) that in turn can lead to catastrophic metabolic failure and cell death.[27]

Peroxynitrite forms whenever superoxide is formed in tissues that also generate NO. NO is formed chronically in the vascular system and in neurons (nerve cells), but under inflammatory conditions, most tissues can form NO owing to increased production 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.

Much of the tissue destruction that follows a heart attack or a stroke is caused by so-called "ischemia-reperfusion damage". ("Ischemia" is a fancy medical term for loss of blood flow.) 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 generation of superoxide – and thus oxidative stress – is dependent on an adequate tissue concentration of oxygen. For reasons that are still not entirely clear, capacity for superoxide production is vastly increased in tissues that temporarily have been deprived of oxygen; NADPH oxidase contributes to this superoxide production, although it is not the sole source. 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 tissues protection.[28-30]

Peroxynitrite also plays a key role in the circulatory collapse and organ damage associated with septic shock[31] – 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[32] (responsible for hundreds of thousands of deaths annually in the U.S. alone). It also contributes to the process of atherogenesis (hardening of the arteries). 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.[33] In fact, not only does this decrease the capacity of this enzyme to produce NO, but it causes the enzyme to start producing superoxide – thereby further increasing peroxynitrite production! Another key vascular enzyme damaged by peroxynitrite is prostacyclin synthetase,[34] which makes a hormone that wards 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.[19] Peroxynitrite is bad new indeed!

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 sometimes amplified in stressed tissues. And other enzymes – notably xanthine dehydrogenase[35] and NO synthase[33] – 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. So medical strategies for coping with peroxynitrite are clearly highly desirable.

Fortunately, a natural metabolite, uric acid (also referred to as urate), formed by the metabolic breakdown of nucleic acids (more specifically, those known as purines), can act as a highly effective scavenger for

peroxynitrite-derived radicals.[36] 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 dehydrogensase, 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 this absorbed xanthine oxidase was the culprit in atherogenesis.[37, 38] 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, but he worried that it would 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.[39] 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.[37, 40] He noted substantial improvements in angina pain; in particular his own consumption of nitroglycerin, used to abort pain during angianal episodes – fell about twenty-fold. Patients who had intermittent claudication – a syndrome in which compromised circulation to the legs makes it difficult and painful to walk for any distance – 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.[41]

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.[42, 43])

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 nerves – 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. So 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. So 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"[37] – 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 arterial linings of people or animals who have atherosclerosis or diabetes.[44] More specifically, they found that these linings – known as vascular endothelium – did a much more effective job of generating protective NO. Further studies demonstrated that 5-methyltetrahydrofolate was restoring the normal function of NO synthase in inflamed endothelial cells.[45-48] You will recall that this function is often compromised, because peroxynitrite damages 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![49] Indeed, folic acid seems to be almost ideal for this role, because in many cells folic acid is concentrated against a gradient, so that its intracellular level can be readily be boosted ten-fold or more when cells are exposed to increased concentrations. 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.

Meanwhile, studies by Dr. An Moens and colleagues at Johns Hopkins and in Belgium are now confirming 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.[50] 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. As Dr. Moens told me (MFM) rather pithily: "We have proved that Dr Oster was right."

A recent rat study by Dr. Moens and colleagues has achieved an even more remarkable result.[51] 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, other studies showed that 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). So perhaps reduced folates have additional protective properties. But, in any case, providing protection from peroxynitrite is enough of a miracle to give high-dose folate remarkable medical potential indeed![52]

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.[53]

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.[54]) 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,[55] 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.[52] 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 – 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.

## Boosting Uric Acid

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 the brain.[52] 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. You'll recall that previously we mentioned the blood-brain barrier. Whereas many of the body's tissues avidly concentrate folic acid against a gradient, so that very high intracellular concentrations can be achieved, 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. So folic acid doesn't appear to have potential as a peroxynitrite scavenger for the brain.

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.[56, 57] Moreover, a high intake of dairy products seems to increase risk for this disorder[58] – 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.[59, 60] High blood urate is also linked to lower risk for multiple sclerosis[61] – and increasing urate levels by feeding mice inosine has been shown to be protective in allergic encephalomyelitis, commonly viewed as a rodent model for human multiple sclerosis.[62] Indeed, pilot studies with supplemental inosine (usually 1-2 grams daily) have yielded promising, though not yet definitive results in multiple sclerosis patients.[63, 64] And 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.[65] So supplemental inosine appears to 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 (trade name Zyloprim). 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 maintaining an alkaline urine, as urate is relatively soluble under alkaline conditions. In any case, it is clear that people thinking of supplementing with uric acid should have their blood uric acid levels checked first, so that an appropriate, safe dose can be administering. In those people whose uric acid levels are near the upper limit of normal, the safe dose may be zero! On the other hand, limited clinical experience in patients with multiple sclerosis – who usually have rather low urate levels – suggest that they have no problem with daily inosine doses of 1-2 grams.

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[66, 67] – 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 sythase from peroxynitrite.

In epidemiological studies, high blood levels of urate tend to correlate with increased cardiovascular risk.[68] 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.[69, 70] 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.[71, 72] Moreover, raising blood urate levels by intravenous infusion was found to *improve* vascular function in smokers and type 1 diabetics[73] – a result which may reflect an important role for peroxynitrite in the arterial disease associated with these disorders.

Inosine supplementation is an attractive option for people who are particularly concerned about neuroprotection. But such supplementation should only be used under physician supervision. A baseline urate levels should be obtained, and a dose prescribed that appears safe in the context of that level (which for some people, will mean no supplementation). Follow-up urate measurements will of course also be prudent. People who are taking inosine would be well advised to concurrently use potassium bicarbonate-enriched water and eat plenty of fruits and vegetables, as this will alkalinize the urine and thus minimize risk for gouty nephropathy.

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.[74] 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![32, 75] Perhaps we should try high-dose folate too!

**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 (as opposed to 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.[76, 77] When oxygenation of the tissue is restored, these pent-up electrons cause a dramatic burst of superoxide production. This phenomenon occurs whenever blood flow to a tissue is temporarily cut off, as during a heart attack or stroke; the tissue destruction that results is known as "ischemia-reperfusion" damage.

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.[78] 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.[78, 79] Thus, mitochondria can act as amplifiers of the oxidative damage triggered by NADPH oxidase. Conversely, the oxidants produced by mitochondria can promote activation of NAPDH oxidase in some cells.[80] Hence, even when a stimulus which promotes oxidative stress is alleviated, vicious cycle mechanisms often can maintain the excess production of oxidants.

**Coenzyme Q10 – Mitochondrial Antioxidant and Bioenergy Cofactor**

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. 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.[81-83] 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.

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.[84-86] 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;[87] 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.[88] 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.[89]

Clinical trials, as well as rodent studies, indicate that supplemental CoQ can have antihypertensive activity. A recent "meta-analysis" – a study which lumps together the results of a number of properly designed clinical trials so as to achieve a more reliable assessment of a drug's clinical utility – 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.[90] 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.[78, 91-95] Injection of minute amounts of CoQ into these brain regions can alleviate mitochondrial damage, thereby preventing or correcting the rise in blood pressure.[78]

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.[96-98] 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;[99-101] this is not surprising given the prominent role of peroxynitrite and mitochondria-generated oxidative stress in ischemia-reperfusion damage..[102] 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.[103-106]

In many neurodegenerative disorders, neuronal dysfunction is characterized by impaired mitochondrial function and increased oxidative stress – suggesting possible utility for supplemental CoQ.[107] 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.[107-110] 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.[111-114]

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.[115, 116] In diabetics, some reports indicate that CoQ can aid vascular function and glucose control in type diabetics, whereas others fail to observe metabolic effects.[96, 117, 118]

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,[119] such supplementation does not appear to prevent the statin-induced muscle damage that occasionally requires discontinuation of these drugs.[120, 121] 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.[122] In dry form, it is inefficiently absorbed; special micellized preparations have improved availability, and thus may be preferable.[123, 124] Furthermore, recent research shows that the ubiquinol, the reduced form of CoQ that functions directly as an antioxidant, is better absorbed than CoQ per se.[125] 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.

## Antioxidant Enzyme Induction

The final major strategy incorporated into 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.[126] Most cells contain a protein known as Nrf2 (make up your own pronunciation!) capable of interacting with special regions of DNA (known as "antioxidant response elements") in a way that greatly increases cellular synthesis 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 the nucleus because it binds to another protein, Keap1 (you don't want to know what that stands for – though we threaten to tell you if you ask!) Keap1 is capable of binding to a number of chemicals that, because of their reactive chemical structures, are capable of attacking DNA and giving rise to mutations. Also, oxidative stress can alter the structure of Keap1.[127] Either sort of structural modification prevents Keap1 from binding to Nrf2, so 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. 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 (in their native forms, or after metabolism) and thereby triggering the phase 2 response – *but that are inherently non-toxic*.

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.[128, 129] 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.[130-132] 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.[133, 134] Lipoic acid has also been used clinically in a range of liver disorders, including mushroom poisoning and hepatitis C.[135, 136] 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.[137-146] 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.[147, 148] 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 phase 2-inducing phytochemicals in a comprehensive antioxidant regimen.

By the way, in case you would like to know, the antioxidant enzymes boosted by a phase 2 response include: heme oxygenase-1 (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. (Don't worry, we won't be giving a test!) Also induced are a number of factors 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 the cell that it is still at risk.

## 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.[149] 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,[150] 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 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.[151, 152] 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.[153-156] 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[157] (though lifelong accumulation of mutations in pre-cancerous 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 Alzheimers and Parkinson's diseases, traumatic brain and spinal cord injury, complications of diabetes, and drug toxicities.[158-164] 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 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.[165-168] Glutathione, which is found in relatively high (millimolar) concentrations in most cells, functions directly as an efficient 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.[169-175] 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 in 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.[176] NAC supplementation has been shown to boost cellular and blood levels of glutathione, both in rodents and humans.[176-178] 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.[179] 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.[180-184] 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.[176, 185-194] 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![195] Rodent studies likewise demonstrate that NAC can confer protection from the flu.[196-198] This protection presumably reflects a key role for oxidative stress in the inflammatory response evoked by influenza infection.[199]

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.[200] 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.)

## Ancillary Antioxidants

We have now described and explained the key components of an **Full-Spectrum Antioxidant Therapy**: reduction of NADPH oxidase activity with PhyCB from spirulina; scavenging of peroxynitrite-derived radicals with high-dose folate and (optionally) inosine; protection of mitochondria with CoQ; induction of

antioxidant enzymes and promotion of glutathione synthesis with the phase 2 inducer lipoic acid, 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.[201, 202]

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.[203] 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 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.[204, 205] 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, tend 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.

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.[206-208] 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.[209-211] A landmark supplementation study organized by Dr. Larry Clark, in which over`1300 people which people 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, observed significant reductions in risk for prostate, colon, and lung cancer in those taking selenium – and a halving of the cancer death rate![212] Closer analysis of the data, however, revealed that the reduction in cancer incidence was confined to those with lower baseline selenium levels.[213, 214] And a further smaller study, in which 400 mcg of selenium daily was given, failed to show an impact on cancer risk.[215] More recently, a large follow-up study, the SELECT trial,[216] 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. 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. But further research will be required to validate this view. These findings underline the fact that rodents treated with large doses of carcinogens may be poor models for spontaneous cancer incidence in humans.

Another essential antioxidant that has received considerable evaluation is **vitamin E**. 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.[217] It was hoped and expected that vitamin E supplementation would decrease cardiovascular risk by decreasing oxidative damage to LDL particles (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.[218, 219] Indeed, there was even a hint in some studies that vitamin E was modestly increasing risk;[220] 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.[221-223] Whether or not this is true, it may be prudent to balance supplemental vitamin E with a comparable intake of gamma-tocopherol. Evaluation of the clinical potential of supplementation with either gamma-tocopherol or "mixed tocopherol" preparations is still in a early stage,[224, 225] though intriguing anti-inflammatory effects in rodent studies – not achievable with alpha-tocopherol - have been reported.[226-229] In any case, it is now clear that supplemental vitamin E is not a "wonder nutrient" - probably 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,

**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.[230] 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.[231-233] (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.[219] 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.[234]

Sooner of 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.

(In passing, it should be noted that, 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.[235] 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.[236-238] Thus high-dose intravenous sodium ascorbate is now being seriously studied as a strategy for shrinking cancerous tumors, without harm to healthy tissues.

On the other hand, the oxidized form of vitamin C – dehydroascorbic acid (DHA) – may have clinically important antioxidant 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.[239] 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.[240-242] 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.[240, 242] Arguably intravenous antioxidant therapy for heart attack, stroke, and various shock conditions could also incorporate PhyCB, high-dose folate, 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.[243-245] Unfortunately, folate – in the guise of folinic acid, a natural metabolic derivative of folate that aids the efficacy or safety of certain cancer chemotherapies – 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** are fat-soluble compounds found in many fruits and vegetables that have scavenging antioxidant activity. **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[246, 247] – whereas this problem hasn't been seen with even high intakes of beta-carotene.

Although carotenoids can scavenge many types of radicals, they are 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.[248] 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.[249] 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.[250, 251] 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.[252-254] A favorable impact of lycopene supplementation on the progression benign prostate enlargement has also been reported recently.[255] 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.[256-258]

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.[259-263] 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.[264] 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;[265] 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.[266, 267] 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.[268] Moreover, there is evidence that peroxynitrite is a mediator of the DNA damage induced by lung cancer tar.[269] So it is conceivable that antioxidant prevention of lung cancer will indeed become feasible – once we employ the right antioxidants.)

A carotenoid structurally related to lutein/zeaxanthin – known as astaxanthin – is found in certain microalgae, and in crustaceans, fish or birds that consume that algae, directly or indirectly. It is what makes flamingoes and salmon pink! Astaxanthin has excellent activity as an antioxidant for cellular membranes and LDL particles;[270, 271] its antioxidant activity appears to be more versatile than that of other carotenoids, owing to the presence of hydroxyl groups that can donate electrons. In a number of rodent studies, oral astaxanthin has shown favorable effects on vascular health and insulin resistance syndrome.[272-275] Moreover, a synthetic, water-dispersible derivative of this carotenoid, which can be administered intravenously, provides substantial protection during simulated heart attacks and strokes in rodents, and may have potential as a drug for use in emergency medicine.[276] However, there has been relatively little clinical research with oral astaxanthin so far, so it is difficult to assess what doses might be protective in humans; the doses employed in rodent studies are proportionately far higher than those provided by currently available supplements (typically 2-4 mg per cap). One intriguing small clinical study suggests that a daily dose as low as 16 mg may have a favorable impact on male infertility, presumably by providing antioxidant protection to sperm.[277] It's hard to assess astaxanthin's utility from epidemiological studies since, aside from diets rich in salmon, most natural human diets are quite low in this compound. Astaxanthin, in some sufficient dose, likely has considerable potential for human health promotion,[278] but the jury is still out until more clinical research has been accomplished.

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. Astaxanthin has considerable potential for protecting cellular membranes, and bears watching.

## Protective "Carninutrients"

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".[279] Vegetarians tend to have lower body stores of these nutrients than omnivores do, and thus they are most likely to benefit from carninutrient supplementation. Of these nutrients, both taurine and creatine can act as antioxidants.

**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,[280, 281] a finding whose credibility is enhanced by the documented utility of taurine in a rabbit model of this syndrome.[282, 283] 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, reducing risk for clot formation.[284, 285] 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.[279] 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.[286]

Taurine levels are also high in immune cells that act as phagocytes – known 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 indeed has anti-inflammatory properties.[287, 288] 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.[289-292] In particular, taurine has been protective in rodent models of atherogenesis, possibly because activated phagocytic cells in the arterial lining (macrophages known as "foam cells") play a pathogenic role in this disorder.[286] Taurine also protects rodents from certain complications of diabetes.[293-297]

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,[298] 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.[299] (This is a bit technical – but, for my biochemist readers, 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.[300] 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.[301]

Although the impact of supplemental creatine on sports performance has received a fair amount of attention from scientists and the lay public, its greatest benefit to health may stem from its neuroprotective potential. 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.[302, 303] It is not clear whether this benefit reflects improved neuron bioenergetics, an antioxidant effect, or both. 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.[304] Of related interest is a recent study in which dietary creatine was found to modestly enhance the average lifespan of aging mice.[305]

In any case, there is reason to hope that creatine supplementation will reduce risk for, and perhaps even aid control of, common neurodegenerative disorders such as Alzheimer's or Parkinson's diseases, and will also reduce the brain damage induced by strokes. 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 does not function as an antioxidant, but it 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, presumably because carnitine levels are already high enough in the skeletal muscle of unsupplemented people to optimize its fat-burning function. 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.[306] 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.[307] 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.[308-310] 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.[311]

**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.[312, 313] 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.[314-316] 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.[314, 316-318] 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.[317, 319-322] 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.[320, 323-326]

There is an additional way in which glycine can act as an antioxidant – specifically in the liver. 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.[327, 328] 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,[317, 318, 320, 323-326] 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.

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.[329-333] This probably reflects the fact that glycine can act as a scavenger for reactive molecules which cause glycation.

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.[315, 334] In rodents, dietary glycine has been shown to slow the growth of tumors, apparently owing to its inhibitory impact on the angiogenic process.[334, 335]

The possibility that glycine might favorably influence vascular health by exerting an antioxidant effect on endothelial cells has been suggested, and merits evaluation.[336]

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. Nonetheless, 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.[337] 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.[338-341] 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.[342-344] 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.[345-350] 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.[351] 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.[352] 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.[353] Overall, there is not compelling evidence that increased iron stores increase diabetes risk via oxidative stress – but we will 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.[354, 355] Thus, there are theoretical grounds for suspecting that increased iron stores may boost cancer risk.[356] 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.[357-361] 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.[362] 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.[363] 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.[364] 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![365] 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.

## Caloric Restriction vs. Mitochondrial Oxidative Stress

As you will recall, mitochondria, the "power plants" of our cells, inevitably produce some superoxide while generating the bioenergy catalysts 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.[366] 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.[367-369] 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.[370-372] Vegan diets of modest protein content tend to be relatively low in methionine,[373] and a fat loss regimen in which aerobic training sessions are nested within daily "mini-fasts" appears to have potential as a sustainable strategy for weight control.[374] 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!

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; 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.

### 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.[375] 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.[376, 377]

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.[378, 379] 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[380] - 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.[381-386] 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.

## Additional Benefits of Nutritional Insurance Supplementation – Focus on Vitamin D

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 some of the other nutrients and phytochemicals which have health-promoting potential as supplementary nutraceuticals:

"Nutritional insurance formulas" are supplements designed to insure adequate or optimal intakes of the nutritionally essential vitamins and minerals.[387] 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.[388-390] 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 about 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.[391, 392] 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. alone die prematurely from cancer owing to suboptimal vitamin D status reflecting inadequate UV exposure.[393] More recently, his work points to a major influence of vitamin D status on survival in people with pre-existing cancer;[394] 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.[395, 396] 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.[397]

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.[398] 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.[399]

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.[400-402] 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.[403, 404]

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![405-414] 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.[415-417] 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.[416]

**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.[418, 419] 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[420] – 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.[421, 422] 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.[423] 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.[424]

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![425, 426] 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.[427] Vitamin K promotes carboxylation of a protein in the vascular wall (MGP) that helps prevent inappropriate calcification of arteries.[428] Epidemiological studies suggest that vitamin K2 may be more effective than vitamin K1 in this regard.[429] 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.[430, 431] 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.[432] 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 undercaboxylated 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.[433, 434] Fortunately, increased intakes of vitamin K promote increased carboxylation of prothrombin in these cancers, so that production of the undercarboxylated growth factor is suppressed.[435, 436] 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.[436, 437] 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.[438] 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.[439-441] 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.[442, 443] 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.

## Benefits of Optimal Mineral Nutrition

The mineral nutrition provided by well-designed nutritional insurance formulas can also provide health protection, particularly for those who may eat too many refined, sugary, or fatty foods. 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. **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;[444-446] 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.[447-450] Good **zinc** status helps to support effective immune function and wound healing, and is especially vital for patients who are critically ill.[451-453] Zinc deficiency tends to amplify oxidative stress, for reasons not yet entirely clear.[454, 455] This may have some bearing on recent reports that good zinc status is associated with lower risk for type 2 diabetes, diabetic complications, and

advanced prostate cancer.[456-459] Moreover, in conjunction with antioxidant vitamins, supplemental zinc has been shown to reduce risk for advanced age-related macular degeneration;[460] oxidative stress is thought to play a key role in this disorder, which is the chief cause of blindness in the elderly.[461] **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. Bioavailable forms of **chromium** such as chromium picolinate appear to have a favorable impact on impaired insulin sensitivity in at least some people[462-465] – Chinese diabetics appear to get particular benefit in this regard[466] – and a recent clinical study has found that this mineral promotes appetite control in women who have carbohydrate cravings.[467] Other nutritional minerals with potentially beneficial health impacts – but which so far have received little study – include **silicon**[468-472] and **boron**.[473, 474]

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.[475, 476] 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%.[477-479] 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.[480] 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 osteoarthritis. There is limited evidence that exposure to strontium in drinking water may aid prevention of dental caries in young people;[481, 482] 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.[483-485] In any case, strontium supplementation appears to have a bright future in preventive health.

**Potassium for Vascular Protection and Bone Health**

Potassium is usually considered an "electrolyte" rather than a mineral. Administering potassium in tablet form is not generally a good idea, as high local concentrations of potassium can be very irritative to the intestinal trant and even cause ulcers. Therefore, potassium is best ingested in potassium-rich foods or drinks. Diets rich in 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.[286, 486-491] It might be reasonable to consider potassium an antioxidant, as moderate increases in blood potassium levels act to decrease superoxide production by the endothelial lining of arteries.[492, 493]

One key benefit of a high dietary potassium intake is that it boosts the capacity of the kidneys to secrete sodium.[494-496] Thus, potassium may act as a functional antagonist of the many adverse health outcomes associated with salty diets – which include not only hypertension and stroke, but also congestive heart failure, osteoporosis, kidney stones, and asthma.[497, 498] There is even suggestive evidence that salted diets may play a permissive role in the genesis of dementia.[499] Epidemiologists should devote more attention to the possibly favorable impact of potassium-rich diets on risks for these disorders.

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.[500-502] 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[503, 504] – 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.[505, 506] 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.

## 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. These types of fats play a key structural role in the brain – and diets poor in omega-3 nutrition have been linked to increased risk for depression and suicide.[507-509] Good omega-3 nutrition appears to reduce risk for so-called "sudden death" cardiac arrhythmias.[510, 511] And 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.[512-516] 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.[517, 518] Strict vegans will be heartened to learn that

long-chain omega-3s derived from algae cultures are now becoming available in supplements – albeit they are considerably more expensive than those from fish oil (at least as long as there are any fish left!)

## More Benefits from Phytochemicals

Many protective phytochemicals can support health by acting as oxidant scavengers, or by boosting cellular expression of antioxidant enzymes. 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.

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.[519] 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).[520-525] 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.[526] 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.[527, 528] There are a few other societies in which hypertension does not develop, but, in all of these societies, food is not salted. 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![529] 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 is planning to introduce flavanol-rich chocolate bars in the future, but it really shouldn't be necessary to get all of that saturated fat in order to get the benefits of cocoa flavanols. Fortunately, flavanol-rich cocoa extracts are now available for use in supplements and functional foods.

**Soy isoflavones** also 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.[530] 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.[531] 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.[532-534]

The blood concentrations of 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.[531] (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. And 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.[531] (Epidemiological studies in Asia have correlated soy-rich diets with reduced risk for breast cancer.) 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.

Another phytochemical employed in this program 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).[535-537] 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.[538, 539] 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.

## Nutrients for Special Needs

For people who unfortunately are already diabetic (whether type 1 or type 2), a special form of the B vitamin thiamine may have considerable potential for preventing the distressing and life-threatening complications of this disorder. Increased intracellular concentrations of thiamine (in its activated form, thiamine pyrophosphate) can markedly activate the enzyme transketolase. In tissues that are targets for diabetic complications (in which cells are highly glucose-permeable), increased activation of transketolase has been found to lower intracellular concentrations of certain glucose metabolites that are excessively high in diabetics, and play a key role in the induction of diabetic complications.[540-543] Thus, it would make a lot of sense to markedly boost thiamine concentrations in diabetics. Unfortunately,

intestinal absorption of thiamine per se is limited by an absorption mechanism that is saturated by normal food levels of thiamine. In other words, if you give high-dose thiamine to diabetics, very little of it will be absorbed.

Fortunately, this problem was solved about 50 years ago by Japanese biochemists who were seeking to develop an improved absorption form of thiamine for use in the treatment of Wernicke-Korsakof syndrome, a type of psychosis not uncommonly encountered in alcoholics who are severely thiamine deficient. They developed a fat-soluble chemical, called **benfotiamine**, which was very efficiently absorbed and, once within the body, was spontaneously transformed to thiamine.[544-546] Benfotiamine proved to be very safe – indeed, in rat studies, it was less toxic than thiamine in extremely high doses – and so has been in use for decades as a safe supplemental source of thiamine activity. Indeed, in the U.S., benfotiamine is readily available over-the-counter as a legal and inexpensive nutraceutical. Studies with diabetic rodents show that benfotiamine has a favorable impact on diabetic kidney damage, heart contractile function, and wound healing.[547-549] Initial clinical studies show that benfotiamine is helpful in diabetic neuropathy, and acts to normalize vascular function.[550-552] In the most recent clinical study with benfotiamine, its use in conjunction with lipoic acid in patients with type 1 diabetes was shown to normalize several markers for glucose-induced damage and boost production of the vascular-protective hormone prostacyclin.[553] The effective clinical dose for these purposes appears to be in the range of several hundred milligrams daily, split over two or three doses per day. In light of current evidence, diabetics should be strongly encouraged to take benfotiamine. When used in conjunction with the comprehensive antioxidant measures included in this program, there is good reason to believe that diabetic complications will be preventable, or at least postponable, to a marked extent.

Another special-purpose nutraceutical with health-promoting potential is **glucosamine**, recommended for prevention and control of osteoarthritis and osteoarthritic pain. Glucosamine is a biosynthetic precursor for the hyaluronic acid in synovial fluid[554, 555] – 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).[556-560] Moreover, two extended controlled studies employing glucosamine sulfate found that loss of knee cartilage was slowed in patients taking the glucosamine.[561-563] 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![564] 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.[565-569] 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.[555]

The inconsistent results of clinical research with glucosamine – the largest, most high profile clinical trial had a negative outcome[570] - have led to some skepticism regarding its efficacy in medical circles. We believe that glucosamine is more than just a placebo for the following reasons:

- 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.[571, 572] The market for glucosamine is big and getting bigger. Supplements that are phony hypes don't tend to have this staying power.

We think there is 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 selling glucosamine to people with osteoarthritis, and getting their feedback, one of us (MFM) is essentially certain that a 3 gram daily dose works better and more quickly than the standard 1.5 gram dose. Indeed, we 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.[573, 574] 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.[575-579] 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.

As we note earlier, there is some reason to suspect that PhyCB might also prove helpful for preserving cartilage in people with osteoarthritis, as activation of NADPH oxidase in cartilage cells (chondrocytes) appears to be a key mediator of the cartilage degradation associated with this syndrome.[580-582] Indeed, administration of a drug which inhibits NAPDH oxidase was shown to prevent suppression of cartilage protein synthesis in a rat model of arthritis.[583] You can also promote cartilage health by keeping your weight down, and by engaging in lower-impact aerobic exercises, such as brisk walking, stair-climbing, and elliptical gliders.

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 our discussion should not be construed as evidence that it is of questionable value. In this brief essay, emphasis has been placed 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.

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