Antioxidants were not created equal

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Although "increasing antioxidants" still lags behind "lowering cholesterol" as commonly accepted health advice, the public's recognition of its importance has grown exponentially over the past decade. Yet, just as reducing serum cholesterol levels is only one of many ways to positively affect cardiovascular disease, and thus an over-simplification, maybe even more so the injunction to increase antioxidants for heart disease, as well as so many other diseases. It really is not the case of "the more, the better", it is very much a matter of how to best go about this. Taking exogenous conventional antioxidants (such as the antioxidant vitamins) quells free radicals on a one-to-one basis and is proving to have little utility, while we have shown that promoting endogenous production of catalytic antioxidants is advantageous. This is because the body's antioxidant enzymes are so much more effective, molecule for molecule, quelling free radicals at remarkably higher rates. Perhaps an apt analogy would be trying to extinguish a fire with a bucket containing a gallon of water (dietary antioxidants) or using a firehose attached to an inexhaustible supply (endogenous **catalytic** antioxidants). This leaves little doubt as to which approach would best put out the "fire" caused by excessive free radicals. Some antioxidants are clearly superior to others.

To explain more in detail, higher eukaryotic aerobic organisms, such as we humans, cannot survive without oxygen and yet oxygen is inherently dangerous to our existence. This is why, oxygen-derived free radicals, the natural consequence of oxygen metabolism, are implicated in numerous diseases and degenerative conditions. Thus, oxygen remains one of the paradoxes of life on this planet: we need to breathe to live, yet ultimately it is proving to be the primary cause of senescence and death.

A few definitions are in order. First, free radicals, the common by-products of aerobic metabolism, are responsible for oxygen toxicity commonly referred to as oxidative stress. Oxidative stress may be loosely defined as that state in which exposure to free radicals or other oxidants represents a challenge to normal function or even survival. It occurs when oxidative balance is upset by increased production of oxidants, or by decreased availability of antioxidants contributing to the pathological dysfunction of the organism. It is now recognized that oxidative stress is associated with more than 100 (1) diseases as well as the normal aging process.

Increasingly, scientists believe oxidative stress plays an important contributory role in the pathogenesis of cellular aging (2), and is associated with heart disease (3), stroke (4), Alzheimer's (5), and cancer (6,7). In nearly all cases it is not clear whether the role is a causative one, or whether the oxidative damage is simply a sequela of other types of tissue injury.

WHAT IS A FREE RADICAL?

To provide a biochemical explanation, a free radical contains one or more unpaired electrons. The reductive environment of the cell provides an opportunity for the univalent reduction of oxygen and these forms of oxygen are reactive, owing to the tendency of electrons to pair. Hydrogen peroxide and the hydroxyl radical are also common reactive oxygen species (ROS). The hydroxyl radical, responsible for oxygen toxicity in aerobic environments, is one of the most reactive free radicals known. There are many sources of free radicals both within and external (environmental) to cells. Oxidants generated by the normal metabolism in the mitochondria are the major source of oxidative stress that accumulates with age. A number of normally functioning enzymes such as xanthine oxidase, cytochrome p450, monoamine oxidase, nitric oxide synthase are also sources of ROS. In the brain, free radicals are produced from the autoxidation of norepinephrine and dopamine. The presence of metals such as iron and copper in an unsequestered form can significantly increase the level of oxidative stress.

The major targets of ROS are biological molecules such as lipids, proteins and nucleic acids. Damaging effects of ROS include the oxidation of low-density lipoprotein which is thought to cause atherosclerosis, DNA damage leading to cancer, the triggering of inflammation, the suppression of the immune system, etc. Lipids are very vulnerable to free radical damage and lipid peroxidation is an indication that oxidative stress exists in our bodies. When lipids are oxidized, cell membranes lose their function, organs fail and organisms die. Markers of lipid peroxidation are the best general indicators of oxidative stress. These products cause structural modification of proteins, inactivation of enzymes, and structural damage to our DNA. These are the serious consequences that result from oxidative stress. Oxidative attack on proteins causes irreversible site specific amino acid modifications which can destroy the function of the specific protein molecule and could mark the protein for proteolysis. Free radical damage to the DNA in the form of deletions, can lead to mutations and other lethal genetic effects.

OXIDATIVE STRESS AND AGING

Now, what truly fascinates us is the interplay between free radicals and antioxidants. The free radical or mitochondrial theory of aging, proposed first by Denham Harman in 1956 (8), may not account for all aspects of aging but has received increasing recognition during the last 20 years. A basic tenet of this theory is that the fundamental changes associated with the aging process are the cumulative result of the ROS which are normal by-products of aerobic life. Mitochondrial metabolism is known to be a major source of superoxide generation, as well as of the non-free radical oxidant hydrogen peroxide, contributing to oxidative stress and aging (9). The consequence of this destructive activity is an inefficient cell and a body we recognize as aged (wrinkled skin, low energy level). A plethora of evidence exists to suggest this same aging process plays a major role in tissue degeneration associated with aging as well as age-associated neurodegenerative conditions. The gradual loss of energy with age is paralleled by a decrease in number of mitochondria per cell, as well as the health and energy-producing efficiency of those remaining mitochondria.

The evidence for free radical/ROS involvement in aging is more correlative than direct. Free radicals and other activated species are so difficult to measure under biological conditions that the evidence for their role in disease is normally indirect. There is increasing evidence for the accumulation over time of damaged DNA and the modification of proteins and other molecules. It is calculated that endogenously generated oxygen free radicals make about 10,000 oxidative interactions with DNA per human cell per day (10). These modifications and damage to such vital molecules would be expected to ultimately lead to deficiencies in normal functions in a global way – **aging**. And this is where the fascination truly holds sway. Although scientists are far from demonstrating the connection, the mere possibility of extending life, "cheating death" as it were, through quelling free radicals engages us at a primal and vital level.

Let's look at this closely. Overexpression of antioxidative enzymes retards the age-related accrual of oxidative damage and extends the maximum life-span in a variety of models. The maximum life span of *Drosophila melanogaster* (fruit flies) was significantly increased by the transgenic overexpression of both superoxide dismutase (SOD) and catalase (11), lending strong support to the Harman theory. Other work in mammals suggested a possible correlation between SOD and/or catalase expression and life span (12,13). Very recently, Schriner *et al*. (14) have significantly extended both median and maximal life span in transgenic mice expressing a catalase targeted to the mitochondria. In view of this work, perhaps the ''free radical theory of aging'' should be renamed as the ''oxidative stress theory of aging'' to be inclusive of nonradical oxidants.

The least contested, extensive animal studies on aging clearly demonstrate that caloric restriction substantially slows the rate of aging. Furthermore, it delays the onset of age-associated diseases. Weindruch (1996) (15) concludes that caloric restriction slows aging primarily by an associated decrease in oxygen free radicals produced by the mitochondria. Restriction of caloric intake lowers steady-state levels of oxidative stress and damage, retards age-associated changes, and extends the maximum life-span in mammals. It appears that cells are continuously exposed to oxidants and the rate of aging is a function of this free radical attack, the inefficiency of our antioxidant defenses and the net damage to the cell.

ANTIOXIDANT ENZYMES: OUR FIRST LINE OF DEFENSE

In the history of human beings, which probably derives from our biology and certainly extends into our sports, the interplay between offense and defense has been decisive and offers a simple but compelling analogy about free radicals and antioxidants. Biological systems protect themselves from the damaging effects of free radicals, the offense, with agents that donate an electron to a free radical and converting them to non radical forms. Molecules that react with oxidants to neutralize them are called antioxidants, the defense.

The body relies upon natural antioxidant enzymes to trap free radicals or oxidants and neutralize and detoxify them. The best studied cellular antioxidants are the enzymes superoxide dismutase, catalase, and glutathione peroxidase and redox active molecules, e.g. glutathione and thioredoxin. Less well studied (but probably just as important) enzymatic antioxidants are the peroxiredoxins and the recently discovered sulfiredoxin. Other enzymes that have antioxidant properties (though this may not be their primary role) include paraxonase, glutathione-S transferases, and aldehyde dehydrogenases. Unfortunately, the levels of the natural antioxidant enzymes don't keep up with the increased oxidative stress associated with aging, allowing free radicals to gain the upper hand.

The failure of supplemental antioxidants in neutralizing ROS has been in the forefront of the medical media lately. While a large body of literature does exist proving that oxidants have a detrimental effect in over 100 (1) disease states and may be at the root of the aging process, it appears to be just a logical fallacy that doing the opposite – treating patients with antioxidants – leads to any significant improvement. For example, even though only oxidized LDL is found in plaque formation, reasonable intake of exogenous stoichiometric scavengers of oxidants fails to significantly inhibit oxidation of the lipids. This, of course, is consistent with the wondrous complexity of biological systems and pathophysiology. Well-known antioxidants such as vitamin E and vitamin C have little or no benefit in improving outcomes for subjects in so many well-designed experiments and the results have been quite disappointing overall. For example, a compelling amount of evidence has led to the "oxidative hypothesis" of atherosclerosis, yet randomized, double-blind, placebo-controlled studies such as the HOPE (16) and HOPE-TOO (17,18) trials have concluded that vitamin E supplementation does not prevent cancer or major cardiovascular events, and may, in fact, increase the risk for heart failure.

A similar situation exists for diabetes, where despite the undeniable presence of substantial oxidative stress, attempts to treat the disease by supplementation with antioxidants have failed to produce any significant improvement. Thus, reasonable intakes of exogenous stoichiometric scavengers of oxidants fail to inhibit lipid peroxidation significantly.

The now conventional idea of popping antioxidants does not work and may even be harmful. The reason? Ordinary antioxidants, such as vitamins C or E, only annihilate oxidants on a one-to-one basis. These supplements, called "consumable" antioxidants are used up and excreted from the body. They are poorly absorbed into the system and may not reach subcellular locations where most oxidative stress occurs. In our own study (19) we, at the University of Colorado Health Sciences Center, have shown a statistically significant **increase** in lipid peroxides in those subjects who self-supplemented with vitamins C and E compared to the control group. The reason for this is still not clear, but the inference can readily be drawn that the amount of antioxidant vitamins required to maintain proper oxidative balance is more than these supplements can actually supply.

But there is reason for hope. An alternative remains, based on scientific evidence, not wishful thinking, to return to the body's natural antioxidant defenses and look for ways to stimulate the body to augment its own antioxidant enzymes through natural means. Lifeline Therapeutics has developed a product that does this – Protandim®. Protandim is a combination of five naturally occurring botanical extracts selected to satisfy two criteria:

1. To induce the production of antioxidant enzymes (superoxide dismutase and catalase); and
2. To inhibit lipid peroxidation.

A recent study (19) has already shown positive results in both areas. In retrospect, perhaps, the success of regular Protandim use should come as no surprise. The endogenous antioxidant defense mechanism is obviously the most efficient way to detoxify free radicals. Under normal conditions the body relies upon natural antioxidant enzymes to eliminate free radicals or oxidants and neutralize them. The antioxidant enzymes SOD and catalase, by virtue of their abilities to catalyze the disproportionation reactions of their substrates, the superoxide radical and hydrogen peroxide, respectively, have an enormous theoretical advantage over exogenous antioxidants that are stoichiometrically consumed. Together, these antioxidant enzymes constitute the first line of defense and repair for the body. Thus, rather than eliminating oxidants one-for-one, these catalysts are much more effective because they destroy the oxidant without being consumed or used up themselves.

In our human clinical trial (19) we have shown that administration of Protandim to healthy human subjects 20 to 78 years of age for four months caused significant induction of the cellular antioxidant enzymes with a significant decrease in oxidative stress. SOD and catalase increased 30% and 54% respectively. Protandim also eliminated the age-related increase in oxidative stress. Thus, modest induction of catalytic antioxidants SOD and catalase produced a much greater decrease in oxidative stress than dietary supplementation with antioxidant vitamins (at many times the RDA) or by consumption of food (20). The antioxidant pathways augmented by Protandim resulted in lowering oxidative stress. This stands in marked contrast to studies where traditional antioxidant supplementation did not result in lowering lipid peroxidation.

Providing antioxidant treatment that gets the body to enhance its own defense mechanisms in the fight against free radicals represents a paradigm shift from the conventional position of consuming dietary antioxidants. This is not to understate the importance of traditional injunctions to eat more fruits and vegetables or even to enjoy a little wine once in a while. Many phytonutrients are helpful, but not necessarily as antioxidants.

On the other hand, positing that a paradigm shift has occurred is not overstating the case. Taking one Protandim tablet a day is clearly a more clinically beneficial means of decreasing oxidative stress. The ultimate effect on disease regression and the ageing process is yet to be seen but having a means at our disposal to effectively reduce oxidative stress at the molecular and cellular levels where such stress begins the cascade of events associated with injury is surely an advancement. Antioxidants are not created equal. Protandim is the proof.

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