

Antioxidants

Part 1

*- time to reconsider
the principles?*

The Benefit of Hindsight

If I had known when I was in practice what I know now about 'antioxidants', I may have achieved far better clinical outcomes in some of those more difficult-to-resolve patient conditions. It was only after I moved out of practice in 2004 following a 30-year career in Nutritional Medicine that I had the time available to independently research the many technical issues that underpin the products we prescribe. Based completely on findings from the scientific literature, what you will read here runs counter to most of the popular assumptions surrounding this subject. It may well challenge your established views on what antioxidants are and what they are not - and on what you can expect them to achieve clinically.

It has also become very clear to me that the term 'antioxidants' does not refer to some amorphous group of biochemical substances all doing the same thing. To state that a patient is taking 'antioxidants' is just as meaningless as saying that the patient is taking 'herbs'.

All 'Antioxidants' were not created equal

'Antioxidants' are all individual in their properties; in their cellular compartmentalisation, in their ability to cross cell membranes, in the speed with which they undergo chemical reactions (kinetics), in their solubility, in their bioavailability, in their interactions with other molecules and in their ability to act as messengers, sending signals which initiate critical processes.

In addition, many such as ascorbate have far more significant roles in human biochemistry than in quenching free radicals. We have tended to assume that because these compounds are capable of quenching free radicals in test tubes, they are doing the same in living cells; this erroneous assumption has led us down many a blind alley in both research and in clinical application.



Although ascorbate as one example, is capable of quenching free radicals (more correctly termed reactive oxygen species and reactive nitrogen species), it is not particularly efficient at doing so at the primary level; there are far more targeted molecules designed to keep oxidative species in proper redox balance. The common practice of megadosing ascorbate at hundreds of times the recommended dietary intake (apart from diverging markedly from a '*natural medicine*' model), is like using a shotgun to hit the *bull's eye* – a scattered, rather wasteful and non-targeted approach. There is a much more targeted solution which I will discuss further on.

Misnomers?

Many compounds we categorise as 'antioxidants' are really molecules with unique bioactivity, quite unrelated to any potential for them to act as free radical 'sponges'. This misunderstanding has largely arisen because these compounds were shown in *in vitro* studies to quench free radicals. It was some time before it was realised that quenching free radicals in a test tube could not be replicated for clinical advantage in a living human being. In the interim, the popular notion that these compounds behave as antioxidants *in vivo* has stuck, a misconception arguably fuelled by industry!

A case in point is Vitamin E which has been extensively researched by Angelo Azzi at Tufts University. Azzi's conclusions can be paraphrased as saying, "Vitamin E is clearly doing something in the body – it is an essential part of the diet and deficiency leads to neurological dysfunction – but whatever it is doing, it is *NOT doing it as an antioxidant*¹! In an earlier paper², Azzi described how α -tocopherol modulates 2 major signal transduction pathways and alters regulation of 5 gene families associated with lipid uptake, cell proliferation and platelet aggregation to define the most clinically significant of its roles.

Why do so many vitamin trials fail?

This change in our understanding of what these molecules do helps us to see why so many studies which have used Vitamin E as a clinical intervention tool have failed. They have failed because they are looking for an outcome related to Vitamin E's supposed antioxidant properties; outside the test tube, these are minimal.

A 2001 study³ looked at the effects of escalating doses of Vitamin E from 200 to 2000 IU per day for 8 weeks on lipid peroxidation in healthy adults. They concluded, "Our results question the potential benefit of the reportedly widespread use of Vitamin E supplementation in healthy individuals." This paper reports on many other studies which similarly showed no clinical effect.

A similar conclusion was drawn in a well-designed study⁴ which investigated the long term effects of supplementation of Vitamin C, Vitamin E and β -carotene for primary prevention of Type 2 diabetes. The conclusion drawn was that *'there were no significant overall effects to show that these supplements in any way prevented the development of diabetes in 8171 women over 9 years'*. One must surely conclude that even though oxidative stress is clearly associated with the cellular dysfunction typical of diabetes, these vitamins simply aren't interacting at this level.



A fundamentally-flawed theory

What these studies and others are showing us is that our basic premise on what these essential substances do biochemically, is fundamentally flawed. We know that diseases like cardiovascular disease, diabetes, kidney disease and neurodegenerative diseases (and dozens more) are underpinned by oxidative stress. We also know that individuals who consume a diet rich in 'antioxidant' nutrients enjoy superior health. We have then assumed that if we dose with the known antioxidants as supplements (*Vitamins C, E, beta-carotene and the plant polyphenols and others*), we will take control of the oxidative stress and reduce disease risk.



The research continues to show again and again that the clinical benefits are just not there. At best, we must conclude that the results are inconsistent. Essentially, we are sending in the wrong man for the job! It is just like calling the electrician to fix your blocked drains – the electrician is a very competent tradesman but drains are not his forte. Whatever benefits a whole food diet confers on the health of individuals, it is clearly much more than the effect of the 'antioxidant' vitamins.

Where the Antioxidant Story Began

In the 1950's following research into the effects of the Hiroshima atomic bomb, Dr Denham Harman, an eminent chemist who later co-founded the *International Association of Biomedical Gerontology*, showed how atoms with an unpaired electron were responsible for initiating a chain reaction of indiscriminate damage throughout human cells; he also showed in his laboratory, that these free radicals could be stopped in their tracks if they encountered 'antioxidants' which *quenched* them.

Following his discovery that excessive free radical activity (or oxidative stress) was closely aligned with disease processes and ageing, it was *assumed* that ingestion of antioxidant vitamins to counter the oxidative burden would automatically quench the excessive free radicals and as a consequence, alleviate disease. Known as '*Father of the Free Radical Theory of Ageing*', he proposed that it was excessive free radical activity which played a significant role in the ageing process. It *seemed* logical at the time to '*put two and two together*'

to conclude that supplemental 'antioxidants' could stave off the ageing process and prevent disease.

This giant 'leap of faith' has never been clinically substantiated. And now, it's possible that we still haven't learned from this; the hype surrounding the alleged anti-ageing benefits of the grape-derived compound *resveratrol* is even less based on evidence. I'll explain why in Part 2 of this article.

What really put antioxidants on the map was the 1969 discovery of the core Antioxidant Enzyme, SOD (Superoxide dismutase) by Duke University Medical School researchers, Drs Joe McCord and Irwin Fridovich. Their landmark paper⁵ describing SOD and its extraordinary clinical potential was the catalyst (so to speak) which spawned a whole industry around 'antioxidants' as supplements. The many products we consider to be *antioxidants* are amongst the most-widely consumed of all supplements. However, the remarkable effects of endogenous SOD and the other Antioxidant *Enzymes* shown by McCord and Fridovich have never been replicated by any of the typical dietary compounds we classify as 'antioxidants'.

Was the research hijacked by commercial interests?

What McCord and Fridovich discovered was that the cell manufactures 3 very powerful antioxidant enzymes, SOD, *Glutathione peroxidase* and *Catalase* and that these endogenous enzymes are catalytic in that they will quench a free radical and then recycle themselves and quench another and another and so on.

What this means is that each Antioxidant Enzyme can quench literally *billions of free radicals per minute*⁶. The reaction rate⁷ at which SOD quenches the Superoxide radical has been measured at 2×10^9 free radicals per second. These same enzymes will continue at this extraordinary rate for 3-4 days before being replaced by other similar SOD molecules. However, as we age or are unwell, endogenous Antioxidant enzyme synthesis is less efficient.

Compare this with any diet-derived antioxidant such as Vitamin C where one molecule can quench just *one single radical*. Period! One single radical vs billions per minute! There is absolutely no comparison between the effects of the endogenous Antioxidant enzymes and the diet-derived compounds available as supplements or in foods.

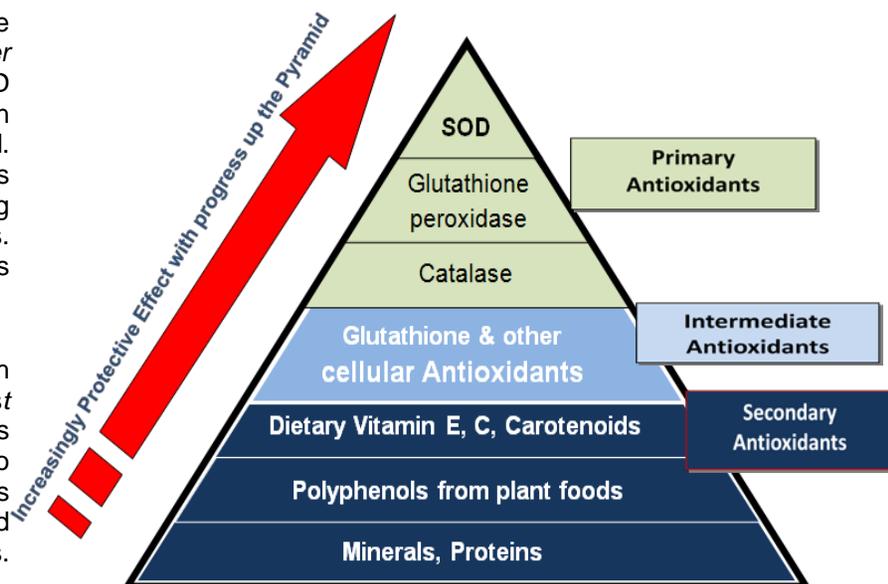
The effect of even a *megadose* of Vitamin C in quenching superoxide is like pouring a glass of orange juice into the Pacific Ocean! It makes virtually no difference! Vitamin C is a valuable and essential nutrient in several key biochemical pathways – but protecting cells (especially DNA) from the damaging effects of free radicals is not one of them!

PRIMARY vs SECONDARY ANTIOXIDANTS

The 3 Antioxidant Enzymes are known as PRIMARY ANTIOXIDANTS, whereas the typical food-derived antioxidants such as Vitamins A, C and E as well as the polyphenols as found in green tea, berries, grapes, curcumin, pomegranate, Coenzyme Q10, Lipoic Acid and Glutathione are all known as SECONDARY ANTIOXIDANTS.

Secondary antioxidants play important biochemical roles but they are certainly not capable of doing what they are so often prescribed to do. Their value will be discussed in depth in Part 2 of this article where we will focus on critical issues such as *bioavailability* and their role in physiological compartments where they exert their most potent effects.

The pyramid representation here clearly shows the hierarchy of radical-quenching ability. Even the extremely important endogenously-synthesised Glutathione can be become depleted when the oxidative burden is high, such as in paracetamol poisoning. Glutathione acts like an 'antioxidant bath' within the cell but like the other secondary antioxidants still quenches



one for one and does not act catalytically as do the Primary Antioxidants.

Preventing DNA damage by upregulating SOD activity

Deep sea divers are known to be susceptible to significant DNA damage because they inhale concentrated oxygen at high pressure. A 2004 study⁸ subjected 20 divers to oxygen at 2 ½ times atmospheric pressure for 60 minutes, after pre-dosing with an orally-bioavailable melon-derived SOD supplement in the 2 weeks prior to the exposure. Quite remarkably, the divers did not demonstrate any DNA damage whatsoever; nor did they display any other markers of oxidative stress.



Vitamins C and E did not prevent DNA damage

A similar study⁹ in 2006 showed that 500 mg Vitamin C and 272 IU Vitamin E pre-dosed for 4 weeks, failed to prevent the DNA damage when 19 men were exposed to hyperbaric oxygen (HBO) for 1 hour.

In addition, under the effect of HBO, there was *significant reduction in plasma Vitamin C and Glutathione levels* as well as significant lipid peroxidation of membranes, demonstrating the very damaging effect of HBO on inadequately-protected human cells.

These 2 studies using the same model clearly reaffirm my earlier contention that diet-derived 'antioxidants' are incapable of dealing with severe oxidative assault on human cells. By comparison, when the cell's endogenous Antioxidant Enzyme Systems can be harnessed, cells are protected against damage.

Interestingly, the melon-derived SOD not only upregulates endogenous synthesis of SOD but it also upregulates both Glutathione peroxidase and Catalase. All three are required to act in concert to completely reduce excessive superoxide radicals to just water and oxygen.

Is the theory overdue for an update?

In the ensuing decades since McCord and Fridovich's seminal work, it has become apparent that the notion that an 'antioxidant' is required wherever there is free radical activity is just too simplistic. What has emerged is that what we term 'free radicals' *are absolutely necessary to provide essential cellular signalling*. In fact, the cell's own defence mechanisms are 'switched on' under the effect of accumulating free radicals. The cell needs to be moderately stressed in order to switch on the genes which code for a range of protective enzymes. However, increasing free radical activity beyond a certain threshold has the opposite effect and leads to oxidative stress.

Nevertheless, the classic view that all 'antioxidants' are good and all free radicals are bad no longer holds true in its purest sense.

Enter the 'Nutrigenomics' era

What we now understand is that free radicals and 'antioxidants' together with a range of other endogenous compounds and food-derived biomolecules are all actors in a cellular script that is constantly changing to adapt to the environment in which the cell finds itself. What's so exciting from a clinical standpoint is that many of the intracellular signalling pathways can be influenced by food-derived biomolecules.

We are now on the cusp of a new paradigm in Nutritional Medicine, the era of 'Nutrigenomics'. Nutrigenomics describes the varied ways in which our food talks to our genes via the many intracellular signalling pathways. Understanding the ways in which food molecules 'switch on' and 'switch off' various genes, allows us considerably more control over our clinical prescriptions. Knowing how to activate the pathways which optimise cellular defence is a core principle for dealing with disease processes of all types. Our goal as Clinicians should be to target the most fundamental causes of cellular dysfunction by addressing pathways as upstream as possible from the clinical effects.



Bioactive food molecules such as the melon-derived bioavailable SOD and the Sulforaphane found abundantly in Broccoli Sprouts are examples of very powerful nutrigenomic substances which interact with other signalling molecules that ultimately upregulate a range of protective compounds. These are foods which 'talk to our genes'. As we learn more about prescribing nutrigenomic substances with powerful clinical 'upstream' effects, we are reminded that these are the medicines of Nature.



Functional Foods as 'serious' medicines

I attribute much of my clinical successes over the years to providing my patients with a strong dietary foundation – not the gimmicky diet trends which remove whole food groups but a *whole food* diet based to a large extent on what epidemiologists have shown to keep certain populations exceptionally healthy. By appreciating that our food contains an entire library of chemicals which interact with our genes, either positively or negatively depending on what foods we choose, it is much easier to convince patients to select foods more wisely.

As an example of the significance of plant compounds, a single generous serve of either spinach, strawberries or red wine has been shown¹⁰ to increase the serum antioxidant capacity to an extent at least equivalent to 1250mg of Vitamin C. The increase was attributed to the effect of the *flavonoids* and *polyphenols* contained in each food. A similar study¹¹ showed the total antioxidant activity of a medium-sized apple was equivalent to 2,250mg of Vitamin C, even though the actual Vitamin C content of the apple was only 10mg. This puts the

relative roles of foods and supplements into a totally different perspective, doesn't it?

If a smoker visited your Clinic seeking advice to prevent the adverse effects of his smoking habit on his health, might you be likely to prescribe typical antioxidants as was done in the following study¹²? Three groups of smokers were given the following daily antioxidant doses over 8 weeks: Group 1 took 200mg α -tocopherol; Group 2 took 200mg α -tocopherol plus 500mg ascorbic acid; Group 3 took 90mg of Coenzyme Q10. Perhaps surprisingly, there were *no significant changes in urinary levels of a DNA degradation product marker in any of the 3 groups*, showing that none of the antioxidants provided any protection at all to the smokers.

By comparison, another study¹³ showed that 300 grams of Brussels sprouts daily for 3 weeks resulted in a significant 28% reduction in the same marker of DNA damage. In Part 2 of this article, we will investigate further the mechanism by which cruciferous vegetables such as Brussels sprouts and Broccoli sprouts can provide significant cellular protection where typical 'antioxidant' supplements can't.

Changing trends

Perhaps we have come the full circle. When I entered clinical practice in 1975, whole food diets had to be a mainstay of treatment because there were so few supplements available. However, we were soon to embrace the era of Megavitamin Therapy – high dose vitamins designed to optimally drive enzymatic reactions.



At the time, I was very much inspired by Adelle Davis, an American biochemist who brought these principles to the fore by taking biochemistry out of the lab and putting into the hands of ordinary people.

Some years later, it was Orthomolecular Medicine, based on Linus Pauling's premise that treatment should be biochemically personalised. (*I don't think we ever had the tools to really personalise the treatment - and of course, we still don't*).

And now, we are again investigating the power of food and the diversity of the bioactive compounds it contains. Although we always knew that fruits and vegetables formed an essential part of a balanced diet, we haven't always known much about the unique and powerful compounds that plant foods in particular, contain. I so often hear it said, 'everyone knows that there is nothing left in our food'. This statement initiated by early purveyors of supplements and perpetrated ever since (often by Clinicians!) is patently untrue.

Although there is some truth in the fact that plants are being grown on mineral-depleted soil and doused in a cocktail of agricultural chemicals, the fact remains that epidemiological studies continue to show that those populations consuming the highest quantities of fruits and vegetables continue to experience better health and less disease.

We shouldn't lose sight of this fact. Whether or not these same plant foods are depleted of nutrients, plants continue to supply us with a rich and varied range of phytonutrients, the substances most likely to 'talk to our genes'.

Christine Houghton

B.Sc., Grad.Dip.Hum.Nutr., R.Nutr., Ph.D. Cand.
Nutritional Biochemist



Following 30 years' practice in Nutritional Medicine, Christine is now engaged in doctoral research at the University of Queensland on 'bioactive plant compounds with significant clinical potential'. She is also the Managing Director of Cell-Logic Pty Ltd, a Brisbane-based company which develops and markets both functional foods and evidence-based niche products designed to optimise cellular function. Her focus on the clinical potential of plant-derived *Nutrigenomic* compounds establishes her as a leader in this emerging discipline.

The copyright to this article is held by:

Christine Houghton

Integra Publishing

www.integrapublishing.com

REFERENCES

- ¹ Azzi A *Molecular mechanism of alpha-tocopherol action* *Free Radical Biology and Medicine* 2007; 43:16-21
- ² Azzi A et al *Vitamin E Mediates Cell Signalling and Regulates Gene Expression* *Ann NY Acad Sci* 2004;1031:86-95
- ³ Meagher EA et al *Effects of Vitamin E on Lipid Peroxidation in Healthy Persons* *JAMA* 2001;285:1178-1182
- ⁴ Song Y et al. *Effects of Vitamins C and E and β -carotene on the risk of Type 2 Diabetes in women at high risk of cardiovascular disease: a randomised controlled trial* *Am J Clin Nutr* 2009;90:429-37
- ⁵ McCord J M & Fridovich I. *Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein)* *J. Biol. Chem*; 1969: 244:6049-55
- ⁶ Trachootham D et al *Redox Regulation of Cell Survival* *Antioxidants & Redox Signalling* 2008;10:1343-1374
- ⁷ Chaudiere J et al *Intracellular Antioxidants: from Chemical to Biochemical Mechanisms* *Food & Chemical Toxicology* 1999; 37:949-962
- ⁸ Muth C et al *Influence of an Orally Effective SOD on Hyperbaric Oxygen-related Cell Damage* *Free Radical Research* 2004; 38(9):927-932
- ⁹ 2006 Bader N et al. *Influence of Vitamin C and E Supplementation on Oxidative Stress Induced by Hyperbaric Oxygen in Healthy men* *Ann Nutr Metab* 2006;50:173-176
- ¹⁰ Ko S-H et al *Comparison of the Antioxidant Activities of Nine Different Fruits in Human Plasma* *Journal of Medicinal Food* 2005;8(1):41-46
- ¹¹ Eberhardt MV et al *Nutrition: Antioxidant activity of fresh apples* *Nature* 2000;405:903-904
- ¹² Prieme H et al *No effect of supplementation with Vitamin E, ascorbic acid or Coenzyme Q10 on oxidative damage estimated by 8-oxo-7,8-dihydroguanosine excretion in smokers* *Am J Clin Nutr* 1997;65:503-507
- ¹³ Verhagen H et al *Reduction of oxidative DNA damage in humans by Brussels sprouts* *Carcinogenesis* 1995;16:969-970