

Antioxidants

Part 2

*- time to reconsider
the principles?*

Reviewing Part 1

One of the most misunderstood topics in Nutritional Medicine is that of so-called 'Antioxidants'. In Part 1, we looked at how 'antioxidants' are classified as Primary and Secondary and highlighted the differences between the typical 'antioxidant vitamins' and the endogenous Antioxidant Enzymes such as Superoxide dismutase, Glutathione peroxidase and Catalase. Where typical 'antioxidants' such as vitamin C, E and beta-carotene are capable of quenching one free radical per antioxidant molecule, the Antioxidant Enzymes can quench several million free radicals per minute¹. This little-known fact may help explain why so many clinical trials using 'antioxidant' vitamins have failed. What's most exciting about the new understanding of the power of the Antioxidant Enzymes is that specific phytochemicals can 'switch on' that part of the DNA which codes for these and other cell-protective endogenous compounds. We are indeed on the cusp of a new paradigm in Nutritional Medicine as we use Nutrigenomics to optimise cellular function and cellular defences.

How do we rate Antioxidants?

A Google search on the term, 'antioxidant' returns around 8½ million websites. A great number of these are dedicated to the sale of various 'antioxidant superfoods', each claiming to be superior to its competitors. So if we were to consider the question, 'how do we rate antioxidants?', the answer really depends on whether you are a clinician, a scientist or a marketer!

Because free radicals have been associated with disease and premature ageing, marketers of 'antioxidant foods' who have sought to capitalise on this situation have struggled to tell their story to consumers because food



(and supplement) regulators limit the claims they can make for such products. How can a marketer promote his antioxidant product to consumers if he can't make any claims for its health-promoting properties? The dilemma was resolved with the introduction of an analytical technique which rated the free radical-quenching capacity of such foods. Enter ORAC.

ORAC – rating 'antioxidants'

ORAC, or *Oxygen Radical Absorbance Capacity* was introduced in 1993 by U.S. researchers at the National Institute of Ageing. One ORAC unit is equivalent to the protection offered by a fixed quantity of a synthetic Vitamin E analogue (Trolox) and is measured as µmol Trolox Equivalent (TE) per 100 grams. The higher the ORAC value, the more free radicals that food could quench in the test tube. Marketers quickly realised that they could promote products such as blueberries and pomegranate for their high ORAC score. Very soon, the 'superfruit' was born – the battle of the ORACs continues to be fought by such fruits as noni, goji, acai, maqui berry and others. So, although health claims as such could not

be made for these products, there was no law to prevent marketers from promoting their products on the basis of their ORAC value. Particularly in the U.S., the ORAC concept caught on quickly and consumers were encouraged to buy high-ORAC foods (usually fruits) accompanied by as many exaggerated claims as the marketer dared to make.

The ORAC concept is less well-known in Australia and most other countries. A 2007 comprehensive table² of ORAC values is available from the United States Department of Agriculture (USDA). On the face of it, it may seem very useful to be able to quote that blueberry has an ORAC value of 6,500 per 100 grams of fruit, that cocoa powder has an ORAC of 80,933 or that raw apple can only boast 3000.

But is this clinically meaningful? Because ORAC is a 'test tube' measurement of antioxidant potential of foods, we can only guess at how many ORAC units daily an individual could aim for. Around 3,000 – 4,000 units daily is a *guesstimate* put forward by the USDA³. It is worth noting that a well-planned diet can exceed 20,000 ORAC units daily but the USDA estimates that the average American consumes only 1670 units per day.

Does ORAC tell us anything really useful?

Using ORAC as described above to obtain an overall indicator of the quantity of plant food in the diet is clinically very useful; however, there are a number of limitations, the most important of these being that some foods such as Broccoli with the greatest potential to enhance cellular defences⁴ aren't particularly impressive on the ORAC scale.

But the most obvious problem with ORAC is that it is based on 100 grams of the food, so that whilst one could readily consume 100 grams of blueberries, it is unlikely that 100 grams of cocoa powder is so palatable! Similarly, herbs and spices tend to rate extremely high on the scale; 100 grams of dried cloves score 314,000 ORAC units and dried oregano scores 200,129 units. Impressive as these high values are, who would eat



such quantities? A glance over Table 1 really puts this into perspective. Worse still, product manufacturers don't always specify what quantity of food was used to calculate the quoted value, so even nutrition-savvy consumers are left wondering!

The marketers have convinced us that expensive and sometimes exotic fruits such as blueberry and

Food	ORAC Value (per 100 grams)	Serve Size	ORAC Value (average serve size)
Pomegranate juice	2341	1 glass (253 mls)	6014
Blueberries	6500	½ cup (72 grams)	4680
Green Tea	1253	1 cup (240 mls)	3027
Orange, Navel	1819	1 medium (140 grams)	2547
Cocoa powder	80933	1 teaspoon (2.7 grams)	2185
Mango, raw	1002	1 whole (207 grams)	2074
Broccoli, cooked	2386	½ cup (80 grams)	1861
Orange Juice	726	1 cup (253 mls)	1837
Potatoes, flesh and skin	1058	1 small (138 grams)	1460
Spinach, raw	1515	½ cup (90 grams)	1364
Lettuce, Cos	3166	¼ cup (4 inner leaves)	1107
Cinnamon, ground	267,536	¼ teaspoon (0.4 grams)	1070
Oats, uncooked	1708	½ cup (40 grams)	683
Tomatoes, raw, ripe	406	1 medium (123 grams)	499
Raisins	3037	12 raisins (12 grams)	364
Lemon Juice	5997	1 wedge (6 mls)	360
Melon, raw	241	1/8 small wedge (55 grams)	133
Extra Virgin Olive Oil	1150	10 mls	115
Ginger, ground, dried	28,811	¼ teaspoon (0.4 grams)	115
Garlic, raw	6665	¼ teaspoon (0.4 grams)	27

Table 1: ORAC Values of common foods, comparing USDA listed values for 100 grams with ORAC values of average serve sizes. Adapted from <http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/ORAC/ORAC07.pdf>

pomegranate are superior because of their high ORAC values but it may surprise you to learn that one red Fuji

apple has a higher ORAC than an average serve of blueberries! In similar vein, a Navel orange, often considered the benchmark for antioxidant intake via Vitamin C is lower in ORAC value than a red apple. The good news is that both are inexpensive and our patients should be encouraged to far eat more fruits and vegetables of *any* variety!

As illustrated in Part 1 of this article, epidemiological evidence shows that the benefits of high intakes of fruits and vegetables are demonstrable whether or not the foods are organic. There is proven benefit from higher vegetable intake^{5,6}, especially for cancer and cardiovascular disease.



Perhaps surprisingly, only 11% of Australian adults and 8% of adolescents consume the recommended 5 serves vegetables and 2 fruits daily⁷. ORAC values can help us to appreciate the direct antioxidant capacity of common foods and to make appropriate selections, without the influence of marketing misrepresentation. What ORAC does not tell us is what happens to the antioxidant potential of the food once it is consumed. As we will see, the story is far more complex than it might seem.

Other limitations of ORAC

Perhaps the most significant limitation of ORAC is that it tells us only about antioxidant capacity of direct antioxidants in contact with the foods in the digestive tract. What happens in the cells really depends on how much of the bioactive compounds find their way into the

cells. And this is because plant bioactives differ vastly in their *bioavailabilities* and the pathways by which they are metabolised.

The foods which tend to rate highest on the ORAC scale contain polyphenols; well-known examples are the *EGCG* in green tea, *resveratrol* in dark grapes or red wine, *ellagic acid* in raspberries and pomegranate, *curcumin* in turmeric. In part because they are large bulky molecules, polyphenols have been regarded as being poorly bioavailable⁸. They also depend on intact colonic microflora for their conversion to bioactive forms. Such poor bioavailability is in the vicinity of 1-8%, a factor which significantly limits their effectiveness in supplements where usually only milligram doses are consumed. And if a patient's microflora are compromised, the polyphenols may have no clinical value at all.

The importance of Bioavailability

Such limited bioavailability can make polyphenols very poor cellular antioxidants. Many of the studies which demonstrate impressive effects of polyphenols are cell culture *in vitro* studies and not human clinical or even animal intervention trials. The doses used in cell culture studies are often so high that it would not be practical to consume them as part of the diet or even as a supplement. Unfortunately, the way the research is reported is not always clear and Clinicians sometimes assume that a reported finding can be replicated clinically. This can unnecessarily tax the patient's budget without providing any clinical benefit.

Many of the overinflated claims surrounding so-called 'super fruits' is because laboratory *in vitro* studies have shown the rapid death of cancer cells when an extract of the fruit is added to a petri dish containing various cancer cells. Polyphenol-rich extracts typically destroy cancer cells on contact. However, the same does not occur in the human body, where the polyphenol must cross several levels of cell membrane from the gut to the cell interior whilst also undergoing metabolic conversion.

Be very wary of products which make cancer claims but don't specify how they obtained their findings; were the research findings based on evidence from an *in vitro* study, an animal study or a high-quality clinical trial?

A 500mg dose administered to a ½kg lab rat cannot be compared to giving the same dose to a 70 kg adult man with a body mass 140 times greater.

Polyphenols and the Mediterranean Diet



Without a doubt, polyphenols are beneficial in human health and the premise on which the *Mediterranean Diet* and the *French Paradox* are based appears to depend on their presence in the

diet. However, the studies on the French Paradox now show that their effect may be primarily due to inhibiting the oxidation of food with which the polyphenols come in contact directly in the gut. Their systemic effects are comparatively much more limited⁹. Small quantities are enough to directly quench free radicals in the gut and are very much less than that required to achieve a systemic effect.

How much is enough?

A preliminary 2010 study¹⁰ investigating prevention of Alzheimer's disease showed that fresh blueberry juice in 9 human subjects enhanced neurocognitive function, improved memory and may have reduced depressive symptoms. At first glance, these findings appear impressive. However, apart from highlighting the very small number of subjects in the trial, other practical limitations are apparent.

Over a 12-week period, each subject consumed up to 621 mls blueberry juice daily depending on body weight, an amount equivalent to 750 grams or 3 punnets of fresh blueberries. At a cost of at least \$4 per punnet for fresh blueberries, this equates to \$84 per week or almost \$4,500 per year. This is not to devalue the study or the value of fresh blueberries in the diet because a balanced diet is a mixture of thousands of food chemicals, not just one food selected for a clinical trial.

Can a blueberry powdered supplement replace the fresh food?

The real lesson here is that there is no way that many functional food supplements can substitute the food itself. Powdered berry products are popular supplements but when one considers that the blueberry study above used 750 grams fresh blueberries daily to achieve its clinical effect, one would require around 75 grams of 100%

blueberry powder daily (*typical drying ratio of 10%*) to achieve the same outcome, and assuming the unlikely situation that there would be no losses of bioactives during processing. Clearly, this is out of the question!

Can you always trust the label claims?

A mixed berry-based supplement available to Australian patients and which claims a high ORAC value lists for each 5 gram serving of the powder approximately 55mg each of 10 different berry powders, a total of 555mg. This is equivalent to only about 5.5 grams of fresh berries! However, the product claims that 2 teaspoons of this powder is equivalent to 2 cups of blueberries!

If we were to assume that all the berries in the product were blueberries (*many of the other varieties in the product are much lower ORAC value*), then the 5.5 grams of fresh fruits equivalent would yield no more than 357 ORAC units, using the USDA Table. (*2 cups of fresh blueberries would score a whopping 18,720 ORAC units!*)



Looking at the ORAC value of a red Fuji apple, it is clear that the 357 ORAC units in the powdered berry product is equal to about one-thirteenth of an apple! Two cups of fresh blueberries? You be the judge!

As Clinicians advising our patients, our critical thinking skills must always be at the ready. When there is the risk that our patients can't always afford our services, it is surely of paramount importance that we are offering them value for money. This is why I have always focused on achieving as optimum a diet as the patient will allow, as a foundation for long term correction of biochemical imbalances. If I can work towards suggesting a diet that provides as many nutrients and phytochemicals as possible, the patient is more likely to be able to afford those really targeted supplements which have regulatory effects in cell chemistry.

As we discussed in Part 1 of this article, 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. 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'*.

Polyphenols and health - unravelling the mystery

Researchers have been puzzled¹¹ for some time as to how polyphenol-containing foods with such poor bioavailability clearly contribute significant health benefits. A January 2010¹² paper helps explain this apparent anomaly by showing that it is the metabolites of the polyphenols which are exerting the biochemical effects systemically. It seems that polyphenols are modified by 2 different mechanisms. In the first, the colonic microflora are essential for substantial modification to bioactive forms; in the second, the polyphenols undergo transformation via Phase 2 Detoxification Enzyme processes such as *glucuronidation* and *sulfation*. Clinicians in Nutritional Medicine are already attuned to the importance of an intact colonic microflora; here is yet another example to confirm this.

Can Resveratrol live up to its media-driven claims?



The highly-promoted study by Australian researcher David Sinclair shows that resveratrol, when administered to mice (*not humans*) retards the ageing process.

What '60 Minutes' in its review of this research failed to highlight is that Sinclair's research was based solely on mice. (*You can watch this online at <http://sixtyminutes.ninems.com.au/stories/liambartlett/640545/forever-young>*)

More so, there was no longevity benefit to 'middle-aged' mice; increased lifespan was only seen when the mice were started on the supplement at birth. The full paper is available free online¹³.

The mice were administered between 100 and 400mg/kg food daily. The authors emphasized, however, that because their findings are based on research in mice, not in humans, they have no immediate and direct application to people, whose health is influenced by a variety of factors beyond those that may be represented in the animal models. (*This fact is somehow 'buried' amongst more emotive commentary*).

Because it is difficult to extrapolate animal results to the human situation, it is hard to estimate what dosage of resveratrol might be required to replicate the animal studies. Examining the data in the Sinclair study, it would appear that around 7500mg resveratrol daily could be required to produce a similar effect in humans to that seen in the mice. Given that available resveratrol supplements currently are in the vicinity of 50-100 mg per tablet, this would equate to an adult requirement for 75 to 150 tablets daily! David Sinclair has been quoted as suggesting a 'dose' of 1000 bottles of red wine daily to achieve the same benefit!

Given the further finding that longevity could only be demonstrated when supplementation was begun at birth, the notion that resveratrol is an 'anti-ageing' supplement for humans is clearly impractical at this stage of the research!

Paradigm shift towards 'Nutrigenomic Medicine'

What appears to be emerging from research into so-called 'antioxidants' is that much of our established theory to explain why plant foods are good for us is fundamentally flawed. There is no doubt that a diet high in plant foods confers significant protection against a host of diseases, not the least of which is cancer. Partly goaded by marketers, we have used the 'antioxidant' theory to explain many of these benefits but we now know that the story is far more involved and we are nowhere near the final chapter!

The mapping of the human genome which was completed around 2003 has opened new avenues for researching the clinical potential of the 10,000 or so known phytochemicals derived from plants. 'Nutrigenomics' is about food chemicals which 'talk to our

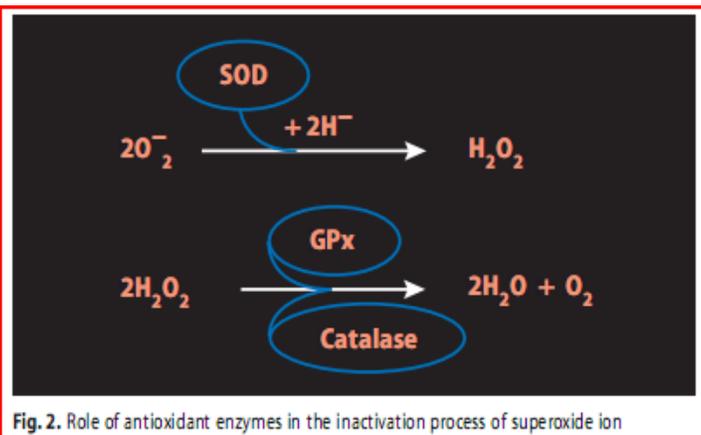


genes', acting as signals to 'switch on' and 'switch off' certain genes as required. Knowing how to activate the pathways which optimise cellular defence is a core principle for dealing with disease processes of all types.

Our goal as Clinicians should be to target the most fundamental causes of cellular dysfunction by addressing pathways as upstream as possible from the observed clinical effects.

Upregulating our own Antioxidant Enzymes

The mitochondria are a major source of the free radical, Superoxide. When the production of Superoxide overwhelms the cell's ability to quench it, oxidative stress results, with widespread damage to organelles and key molecules a destructive consequence. Fortunately, cells are equipped with a series of antioxidant defences to combat such damage; the 3 Antioxidant Enzymes, Superoxide dismutase (SOD), Glutathione peroxidase and Catalase are targeted exactly to regulate superoxide and associated reactive molecules.



A French research group in the late 1990s developed and patented a melon-based extract with the specific ability to 'switch on' the genes which code for SOD and the other 2 antioxidant enzymes. Just 250mg of this encapsulated product has been shown to significantly upregulate these enzymes¹⁴. Several clinical studies have confirmed its clinical efficacy^{15,16}. Table 1 shows that melon has a low ORAC value and yet this melon extract is capable of inducing the antioxidant enzymes

which can quench literally 'millions of free radicals per minute' compared to other foods with high ORAC levels which can quench just one free radical per phenol group.

So if we used ORAC as our guide to determine antioxidant status in the cell, we would draw the wrong conclusion! Similarly, cruciferous vegetables and especially Broccoli rate modestly on the ORAC scale and yet are the only vegetable family with proven cancer-protective properties¹⁷!

Two of the most powerful nutrigenomic food compounds which promote cellular defences, high-SOD melon and broccoli-derived Sulforaphane don't come to the top of the ORAC list. Really confusing, isn't it? And all of this is made worse because your patients are constantly accessing the internet and being influenced by all sorts of half-truths – or worse!



Sulforaphane – 'switching on' cellular defences.

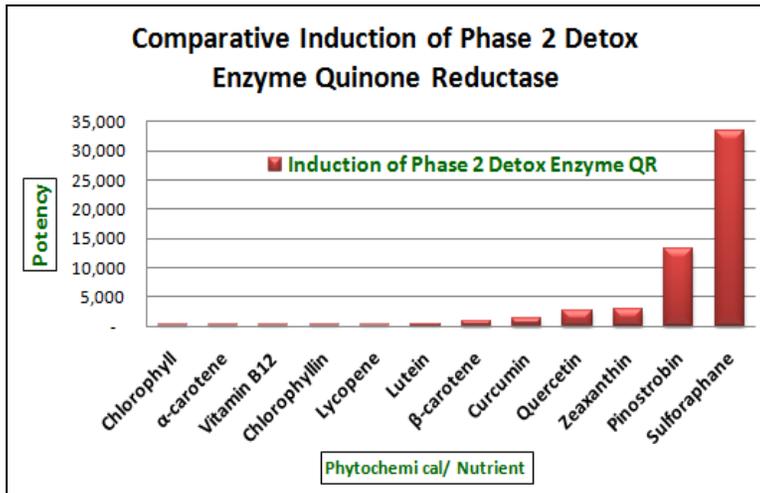
The key bioactive molecule of the cruciferous vegetable family is Sulforaphane and it is derived in highest amounts from Enzyme-active Broccoli sprouts. This remarkable molecule can 'switch on' around 200 genes related to the cell's own defence system. Amongst these is Glutathione, an essential molecule which contributes significantly to the cell's redox balance.

Why struggle with ineffective glutathione supplements when you can utilise Sulforaphane to upregulate the cell's own synthetic pathways?

Most powerful inducer of Phase 2 Detox Enzymes

At the same time, Sulforaphane upregulates the genes which code for the Phase 2 Detoxification enzymes; in fact, *Sulforaphane is the most powerful inducer of this pathway*¹⁸. Sulforaphane's bioavailability has been measured to be around 80% so that the effects attributed to it can occur at manageable dietary levels. Compare this to the polyphenols where comparatively large quantities are required to achieve measurable clinical effects.

Sulforaphane also induces synthesis of other cell-protective compounds such as *Thioredoxin*, *Haemoxygenase-1*, *Ferritin* and *Metallothionein*. At higher cellular concentrations, Sulforaphane influences other cell-protective functions such as apoptosis, angiogenesis, metastasis, cell cycle arrest, HDAC inhibition and more.



Enzyme Activity is critical

Do check that the broccoli sprout powdered product you select is enzyme-active¹⁹. Without the essential *myrosinase* enzyme, there is virtually no Sulforaphane Yield and without this, intended cellular changes cannot occur. Avoid products labelled as 'Broccoli Sprout Extract' no matter how concentrated the extract may appear to be; the very extraction process destroys the *myrosinase* enzyme! A number of other products are mislabelled, quoting Sulforaphane Potential (a *calculated value*), not Sulforaphane Yield (an *assayed value*). Some claim 'high glucoraphanin' but without active *myrosinase* enzyme present, will not produce any sulforaphane when ingested.

The ideal Broccoli Sprout product will be or contain a 100% whole enzyme-active dried sprout powder, optimised for its Sulforaphane Yield. Specialised technology is required to process fresh broccoli sprouts so that the endogenous Sulforaphane inhibitor (ESP) is deactivated whilst retaining the *Myrosinase* enzyme. A superior-quality product can be provided in gram serve sizes once or more daily as required. By a strange quirk of Nature, broccoli sprouts may be one of the few foods where 'fresh' may not be best!

Critically Evaluating Products

On the one hand, it is clear that some quite remarkable phytochemicals have become available to Clinicians in the last decade or so. Such compounds exert powerful effects at the cellular level, translating into positive clinical outcomes.

But on the other hand, selecting the appropriate grade of product and at the dosage required to replicate the clinical trials requires careful consideration. Blueberry extract, Resveratrol and Sulforaphane are just three examples of well-known bioactive ingredients which may not be clinically efficacious in some supplemental forms. Don't believe everything you are told at face value; do your own research!

What have we learned?

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. Both compounds are reliant on enzyme activity for their function and so the way they are processed, stored and dosed is critical for optimal function.

Scientific journals now abound with published papers validating what Hippocrates and others taught more than two thousand years ago; that 'Nature's Medicine Chest' has been an integral part of our evolution on this planet. Processing to more convenient forms can compromise the efficacy of these plant substances.

More than ever, a Clinician must critically evaluate each potential prescription to ensure that the product at hand can deliver what the clinical trials have demonstrated.

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