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Antioxidant Defence Mechanisms: From the Beginning to the End (of the Beginning)

BARRY HALLIWELL

Department of Biochemistry, National University of Singapore, Kent Ridge Crescent, Singapore 119260, Singapore

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When life first evolved on Earth, there was little oxygen in the atmosphere. Evolution of antioxidant defences must have been closely associated with the evolution of photosynthesis and of O_2-dependent electron transport mechanisms. Studies with mice lacking antioxidant defences confirm the important roles of MnSOD and transferrin in maintaining health, but show that glutathione peroxidase (GPX) and CuZnSOD are not essential for everyday life (at least in mice). Superoxide can be cytotoxic by several mechanisms: one is the formation of hydroxyl radicals. There is good evidence that $OH^{\bullet}$ formation occurs *in vivo*. Other important antioxidants may include thioredoxin, and selenoproteins other than GPX. Nitric oxide may be an important antioxidant in the vascular system. Diet-derived antioxidants are important in maintaining human health, but recent studies employing "biomarkers" of oxidative DNA damage are questioning the "antioxidant" roles of β-carotene and ascorbate. An important area of future research will be elucidation of the reasons why levels of steady-state oxidative damage to DNA and lipids vary so much between individuals, and their predictive value for the later development of human disease.

Keywords: Oxygen, antioxidant, superoxide dismutase, hydroxyl radical, glutathione peroxidase, 8-hydroxyguanine, thioredoxin, selenium, DNA repair, anaerobe, β-carotene, ascorbate, iron, Fenton chemistry, nitric oxide, peroxynitrite, transgenic animals

INTRODUCTION: A CASE OF AIR POLLUTION

When life first evolved on the Earth it did so under an atmosphere very different from that which we know today, in that there was virtually no oxygen. Hence the first organisms that evolved on the Earth were what we would now call anaerobes. Oxygen first began to appear in significant amounts in the atmosphere between 2 and 3 billion years ago when some cyanobacteria developed the ability to split water.[1] They did this to obtain energy and reducing power to drive metabolic reactions such as converting CO_2 into carbohydrates. Like many new chemical processes, photosynthesis was accompanied by pollution As a by-product, the cyanobacteria (and later on the higher plants) produced a toxic mutagenic gas. Their liberation of oxygen is perhaps the worst case of air pollution ever recorded on this planet. Present day worries about traces of ozone and oxides of nitrogen pale into insignificance when compared with the impact that the release of oxygen must have had on the life forms that were present at the time.

Adaptation

As oxygen began to appear in the Earth's atmosphere, living organisms had two strategies. Some organisms remained anaerobes and became restricted to environments that the oxygen did not penetrate. They were presumably the predecessors of present day anaerobic bacteria. It turned out that the organisms that have been more successful in evolution were those organisms that evolved protection against oxygen toxicity. They evolved **antioxidant defences** to protect themselves against oxygen toxicity, so that they could tolerate oxygen. Some of those organisms then went on to evolve **aerobic respiration**. The use of electron transport chains with oxygen as a terminal electron acceptor allows oxidation of food materials with much greater ATP yield. Hence the evolution of aerobic respiration is intimately connected with the evolution of antioxidant defence. If we had stayed anaerobic we would never have developed into complex, multicellular organisms. Antioxidant defences of one kind or another probably have an ancient evolutionary history.

Oxidative Stress in Plants

Humans have to breathe 21% oxygen, the current atmospheric level. However, consider the poor plants producing this toxic gas at high levels. For example, an illuminated chloroplast is exposed to an oxygen concentration of 100% and suffers considerable oxidative damage.[2–4] A good place to study antioxidant defence mechanisms is to look at plant tissue.[3,4] Green plants have multiple antioxidant defence mechanisms, including tocopherols, flavonoids, other phenolic compounds, carotenoids and high levels of ascorbic acid.[2–6] Plants do not make ascorbate because humans need it; they make ascorbate because it is a very important antioxidant defence for them and chloroplasts and leaf fluids have millimolar levels of it.[4,7]

We know that eating fruits and vegetables is good for us and it is often proposed that the multitude of antioxidants in plants are a contributor to this beneficial effect. However, these antioxidants are in the plant for the plant's benefit, not for our benefit. We must not assume that because carotenoids and flavonoids are antioxidants in the plant they are necessarily antioxidants in the human body. That is a point that I will return to later.

WHY IS OXYGEN TOXIC?

Credit should first be given to Rebecca Gerschman, Dan Gilbert and their colleagues[8] for suggesting that oxygen is toxic because it makes free radicals. Irwin Fridovich and Joe McCord put a specific free radical in the spotlight, superoxide.[9,10] The superoxide theory of oxygen toxicity states that oxygen is toxic because some of it is metabolized to make superoxide radical. The bulk of oxygen we breathe in is used in oxidative phosphorylation, some is used for oxidases such as cytochromes P450 and monamine oxidases and even smaller amounts of O_2 make free radicals and other reactive species. We make superoxide and these other species, both accidentally (e.g. by autoxidation reactions) and also deliberately, e.g. for phagocyte killing mechanisms and perhaps intercellular signalling.[11,12]

Why is Superoxide Toxic?

Superoxide has an interesting chemistry, because although it is often called a reactive oxygen species, it is not very reactive in aqueous solutions.[13] To put it another way, it is selective with what it reacts. For example, one target that is attacked by superoxide is the enzyme aconitase, a key enzyme of the Krebs cycle in mitochondria and also an enzyme involved in iron metabolism.[14–16] Aconitase has an iron sulphur cluster at its active site, and enzymes of this type may be important targets of direct attack by superoxide.[14] That apart, on the whole superoxide does not react with many things.[13] It does not appear to react at significant rates with DNA,

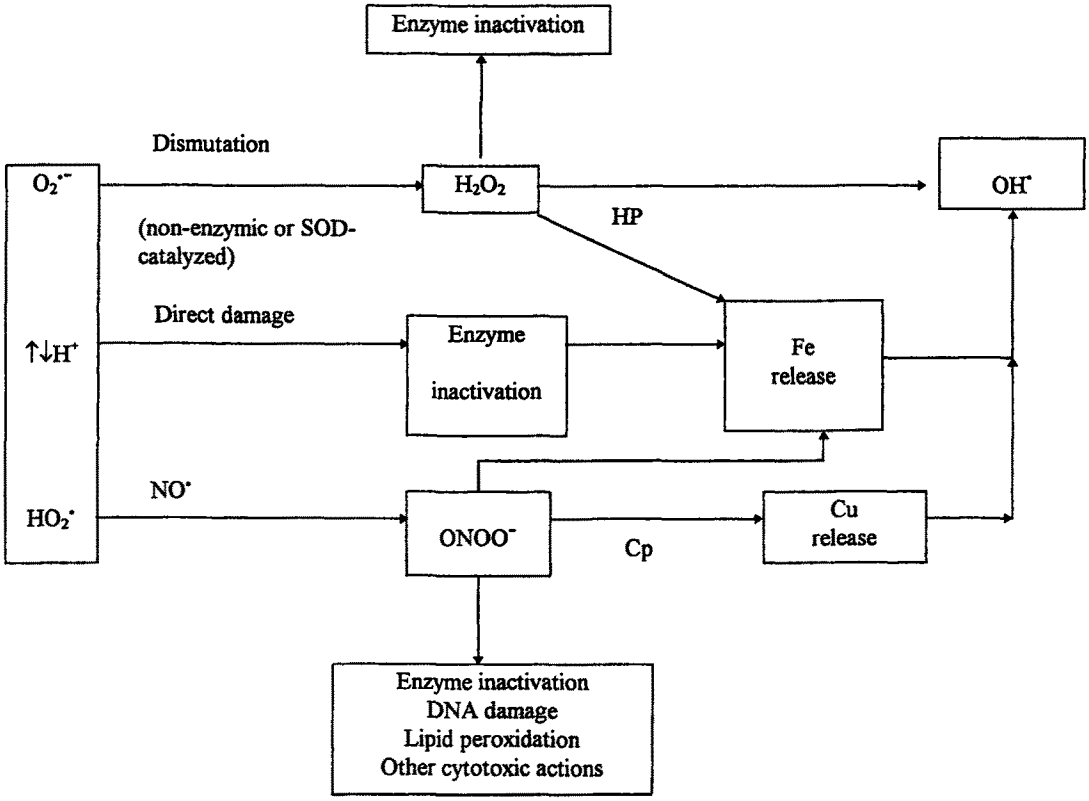

FIGURE 1 Mechanisms of superoxide-dependent damage to biomolecules. Superoxide can react directly with some biomolecules.[14] Its protonated form, $HO_2^{\bullet}$, is more reactive and can oxidize polyunsaturated fatty acids, although it has not been shown to be capable of attacking membrane lipids.[13] Superoxide dismutation produces H_2O_2, which can exert some direct toxic effects and can be a precursor of $OH^{\bullet}$. Release of iron ions from iron–sulphur clusters (by $O_2^{\bullet-}$ or $ONOO^-$) or from ferritin (by $O_2^{\bullet-}$) and from haem proteins (HP) by H_2O_2 can provide the iron needed for Fenton chemistry.[14,18,20] Peroxynitrite is a powerful nitrating, nitrosylating and oxidizing species under physiological conditions[17] and can also displace redox-active copper from caeruloplasmin (Cp).[19]

phospholipids, or proteins.[2] Much of the toxicity of superoxide is thought to be due to its conversion into more damaging species (Figure 1), including peroxynitrite[17] and hydroxyl radical.

The Reality of Hydroxyl Radical

There has been much debate about how important hydroxyl radical is, whether it is really formed in Fenton reactions or *in vivo*. It is thus pertinent to review some of the evidence that it *is* formed.

In 1932 Bray and Gorin proposed that a ferrous salt plus hydrogen peroxide produced a ferryl species (FeO^{2+}), and not the hydroxyl radical ($^{\bullet}OH$), and that ferryl rather than $OH^{\bullet}$ was responsible for most of the damage seen (reviewed in [20]). This old concept has been represented as novel multiple times in the past 25 years. Much of the evidence against the formation of $OH^{\bullet}$ in Fenton systems has been based on the idiosyncratic behavior of a variety of "$OH^{\bullet}$ scavengers" added to Fenton systems damaging biological molecules. Often, however, iron ion-binding, both weak and strong, dictates site-specific reactions that over-rule established second-order rate constants for attack of $OH^{\bullet}$ upon molecules added to Fenton reactions.[20,21] Other arguments against formation of $OH^{\bullet}$ have been based on the fact that the end-products expected when biomolecules were added to

Fenton systems were not "typical of $OH^{\bullet}$". Walling[22] showed decades ago that iron salts influence the fate of the primary radicals generated by attack of $OH^{\bullet}$ on molecules, so that the end-products will not be the same as for radiation-generated $OH^{\bullet}$. Ferryl species could conceivably be intermediates[21] in $OH^{\bullet}$ generation, e.g.

$$Fe^{2+} + H_2O_2 \rightarrow [\text{oxo-iron species}] \rightarrow Fe(III) + OH^{\cdot} + OH^{-}$$

but their formation in "simple" biological Fenton chemistry has never been clearly demonstrated by classical chemical methods (although haem ferryl species are well-established).

What about *in vivo*? Attempts to trap $OH^{\bullet}$ can be confounded by the propensity of this radical to react virtually instantaneously with whatever is present at its site of formation. Spin-trapping methods have been useful in detecting $OH^{\bullet}$ from cells and perfused organs,[23] but have been less successful *in vivo*, although techniques are improving fast. Aromatic hydroxylation methods[24,25] have been used *in vivo* and data are consistent with $OH^{\bullet}$ generation,[26–31] although more confirmatory evidence is required.[25]

One of the systems used to detect $OH^{\bullet}$ is to allow it to react with the amino acid phenylalanine. Hydroxyl radical, being indiscriminately reactive, adds on to the aromatic ring and makes intermediate radicals which then become converted into 3 products, a 2-hydroxylated product (*ortho*-tyrosine), a 3-hydroxylated product (*meta*-tyrosine) and a 4-hydroxylated product (*para*-tyrosine).[32] Table I shows data from an experiment in which blood or synovial fluid was taken from patients with active rheumatoid arthritis, a severe chronic inflammatory disease, and immediately dropped into a solution of phenylalanine. There is immediate formation of products characteristic of attack of $OH^{\bullet}$. High levels of *para*-tyrosine are present because it is a normal metabolite, but the formation of *ortho*- and *meta*-tyrosine is not observed if saline replaces the phenylalanine solution. If we take blood from healthy subjects and drop it into phenylalanine, no *ortho*- or *meta*-tyrosines can be detected. Hence, at least under these reaction conditions, body fluids from patients with rheumatoid arthritis are capable of making $OH^{\bullet}$. It should be noted that $ONOO^{-}$ can also lead to hydroxylation of salicylate and phenylalanine,[25] probably because it decomposes to form $OH^{\bullet}$.[33–35]

Another way of demonstrating $OH^{\bullet}$ formation is to examine the pattern of chemical damage that this radical causes to endogenous biomolecules. Radiation chemists showed many years ago that

TABLE I Aromatic hydroxylation of phenylalanine employed to detect hydroxyl radical formation in body fluids from patients with active rheumatoid arthritis

Subject	Date sampled	Tyrosines (μM)					
		In synovial fluid			In blood		
		o-	*m*-	*p*-	*o*-	*m*-	*p*-
Patient 1	April'92	2.0	2.0	78.0	2.0	1.0	73.0
	May'92	1.0	2.0	61.0	1.0	2.0	54.0
	June'94	2.7	1.6	108.0	3.0	2.0	110.0
Patient 2	April'92	4.0	1.0	84.0	2.0	1.0	65.0
	June'93	0.5	0.5	84.0	0.2	0.3	65.0
Patient 3	June'92	0.9	3.5	66.5	1.0	2.5	60.0
	Nov'92	1.0	2.5	60.0	2.6	0.8	39.0

Blood or synovial fluid were aspirated and immediately dropped into solutions containing phenylalanine. Formation of *ortho*- and *meta*-tyrosines is suggestive of $OH^{\bullet}$ generation. Neither product was observed if saline replaced the phenylalanine solution although *para*-tyrosine a normal metabolite, was still present. Data abstracted from.[32]

when hydroxyl radical is formed near DNA it will attack all the DNA bases.[36] For example, from guanine and adenine it will make hydroxylated and ring-opened structures, and it will attack the pyrimidines also. As far as is known, no other free radical species generates such a wide variety of end-products in DNA. If DNA isolated from a cell or a tissue shows this pattern of damage, it is evidence consistent with the proposal that the damage has been caused by $OH^{\bullet}$. When H_2O_2 is added to isolated mammalian cells, DNA strand breakage occurs, despite the fact that H_2O_2 does not react with DNA. However, DNA isolated from cells treated with H_2O_2 shows the pattern of base modification typical of attack by $OH^{\bullet}$, suggesting that the DNA damage is caused by the formation of hydroxyl radical in the nucleus.[36,37] If cells are pre-incubated with metal ion chelating agents and H_2O_2 then added, there is less DNA damage, which suggests that the $OH^{\bullet}$ formation involves Fenton type chemistry.[38]

ANTIOXIDANT DEFENCES

Let us now turn to antioxidant defences. The first thing known, is that they are not completely efficient. If any aerobic organism is exposed to elevated oxygen levels, overt damage occurs.[39] This shows that antioxidant defences may cope with 21% oxygen but cannot seem to cope with more. Upregulations of defences in relation to hyperoxia may occur in animals, but generally serve to delay rather than to prevent damage.[40] The developing ability to measure oxidative damage *in vivo* has shown that such damage occurs even under normoxic conditions, and aerobes rely on repair systems (especially for DNA) to back up the inadequacies of antioxidant defences.[2]

Enzymes

The key antioxidant defences in the human body are usually thought to include the superoxide dismutase enzymes,[41] the mitochondrial MnSOD and the largely cytosolic CuZnSOD. Because superoxide dismutases make H_2O_2 then one must have enzymes to remove it, the most important of which in humans is usually thought to be glutathione peroxidase.[2] But could we be missing something? One way of gaining information about how important these enzymes are is to examine animals that lack them. A transgenic mouse lacking MnSOD (the mitochondrial enzyme) is a sick animal. Most $MnSOD^{-}$ mice die soon after birth with lung damage; those animals that survive suffer severe neurodegeneration.[42,43] Clearly mitochondrial manganese SOD is a very important enzyme, at least for mice. This is consistent with the common view that the most important source of $O_2^{\bullet-}$ *in vivo* is the mitochondrial electron transport chain.

What happens in mice that lack copper–zinc superoxide dismutase? Such mice appear phenotypically normal. They are not completely normal, e.g. they have problems with reproduction.[44,45] Nevertheless, for day to day life mice can survive without CuZnSOD. This does not mean that CuZnSOD is unimportant, what it means is that if you knock it out, the organism can adapt, and/or something else can take over the function of CuZnSOD. The gene is missing from the embryonic stem cell, it is missing during embryonic development and it is missing from birth onwards, so that the system has plenty of time to adapt. By contrast, adaptation to a lack of MnSOD is clearly not possible.

Similarly, a mouse that does not have the "classical" selenoprotein glutathione peroxidase has a normal phenotype.[46,47] Again, it follows that this enzyme is not essential for everyday life, which does not mean it is unimportant, but rather that the system can adapt to survive without it and/or there are other components that can perform an equivalent function. However, GPX^{-} mice are more sensitive to toxins that generate free radicals, such as paraquat,[46] and their hearts are more sensitive to hypoxia-reperfusion injury.[48]

The properties of $CuZnSOD^{-}$ and GPX^{-} animals suggest that there may be additional

antioxidant systems. For example, thioredoxin has been known for years to play an antioxidant defence role in plants,[49] and evidence is now accumulating that thioredoxin is an important constituent of antioxidant defence systems in animals.[50,51] It is often assumed that the symptoms caused in animals by selenium deficiency are due to lack of glutathione peroxidase. However, the major selenium-containing protein in human plasma is selenoprotein P, which has been suggested to exert antioxidant properties itself.[52] Thioredoxin reductase is also a selenoprotein.[53]

Metal Ion Sequestration

Iron and copper are powerful promoters of free radical reactions and hence their availability in "catalytic" forms is carefully regulated *in vivo*.[54,55] How important is this sequestration? One can apply the same criterion used above: what happens in mice lacking transferrin? Such a mouse has been described: not a transgenic animal, but one with a spontaneous mutation affecting splicing of the transferrin gene.[56,57] These mice die unless periodically injected with transferrin. One can therefore argue that transferrin is more important to everyday life than CuZnSOD or GPX. As in $MnSOD^-$ mice, transferrin deficiency affects the nervous system.[58]

Nitric Oxide

Another antioxidant defence that is frequently neglected is nitric oxide. Nitric oxide is a free radical. Rather like superoxide, it is selectively reactive: it does not react with most biomolecules. However, it reacts very fast with other free radicals (Table II). For example, nitric oxide reacts with $OH^\bullet$ with a rate constant of 2×10^{10}, and with peroxyl radicals with rate constants $> 10^9\,M^{-1}\,s^{-1}$. Hence nitric oxide is a very good scavenger of other free radicals. As a result of its ability to scavenge peroxyl radicals, $NO^\bullet$ is a good inhibitor of lipid peroxidation,[59,60] and it may be that atherosclerotic lesions would benefit from having more nitric oxide to slow down lipid peroxidation[59] and to exert other beneficial effects.[60] I suggest that most of the free radical chemistry of nitric oxide is beneficial to us, although this view has been somewhat eclipsed by a focus on the toxicity of peroxynitrite,[17] the product of reaction of $O_2^{\bullet-}$ with $NO^\bullet$.

Diet-derived Antioxidants

It is widely agreed that in Western countries the health of the population would be improved by eating more fruits and vegetables,[62] but no-one is sure exactly why: there are many constituents of fruits and vegetables that could protect against various diseases. Persons who eat plenty of fruits and vegetables tend to have higher plasma and tissue levels of antioxidants. For example, Gey[63] pointed out that plasma vitamin C levels above about 35–60 μM, an α-tocopherol : cholesterol ratio of > 4.8–5.6 μmol/mmol and β-carotene above about 0.4–0.6 μM are associated with lower risk of myocardial infarction. This kind of evidence does not mean that ascorbate, vitamin E and β-carotene

TABLE II Some rate constants for reaction of nitric oxide with other free radicals

Radical	Rate constant ($M^{-1}\,s^{-1}$)	Reference
Superoxide/$HO_2^\bullet$	$> 10^9$	*Free Rad. Res. Commun.* **18**, 195–199 (1993); *Free Rad. Biol. Med.* **19**, 505–510 (1995)
Peroxyl	$> 10^9$	*Biochem. Biophys. Res. Commun.* **195**, 539–544 (1993)
Tyrosyl	$> 10^9$	*Biochem. J.* **310**, 745–749 (1995)
Tryptanophyl	$> 10^9$	*Biochem. J.* **310**, 745–749 (1995)
Ethanol	$> 10^9$	*J. Am. Chem. Soc.* **116**, 11465–11469 (1994)
$CO_2^{\bullet-}$	$> 10^9$	*J. Am. Chem. Soc.* **116**, 11465–11469 (1994)
$OH^\bullet$	$> 10^{10}$	*J. Phys. Chem. Ref. Data* **17**, 513 (1988)

are protective against cardiovascular disease, simply that a diet that achieves these plasma levels is protective. Similarly, subjects who have high plasma β-carotene levels from diet have a lower risk of developing lung cancer if they smoke cigarettes.[62] This has nothing to do with β-carotene, as shown by intervention studies.[64]

It is also known that most antioxidants can behave in different ways *in vitro*, depending on the assay system.[65] For example, ascorbate has many important antioxidant properties, but when mixed with transition metal ions it is usually pro-oxidant *in vitro*.[66] Does this ever have any physiological significance? If metal ions are normally always safely sequestered in "non-catalytic" forms (see above) probably not. But are they always safely sequestered? This topic has recently been addressed by Rehman *et al.*[67] who supplemented healthy human volunteers with mixtures of $FeSO_4$ and ascorbate. No evidence for increased peroxidisability of LDL was obtained.[68] However, measurements of oxidative DNA damage gave some surprising answers.

There are several ways of measuring oxidative DNA damage.[69] The most commonly used biomarker is 8-hydroxylated guanine, measured as the base (8-hydroxyguanine, 8-OHG) or as the nucleoside (8-hydroxydeoxyguanosine, 8OHdG) Rehman *et al.*[67] measured levels of 8OHG in white cells of blood from healthy human subjects given 60 mg of vitamin C and 14 mg of ferrous sulphate daily, levels close to the recommended daily intakes of these substances. After 6 weeks of daily supplementation, there was no significant change in levels of 8-hydroxyguanine, but by 12 weeks levels were significantly decreased (Table III). By contrast, the sum of all the DNA base damage products measured had increased at 6 weeks (the first sampling point) but had normalized by 12 weeks, even though supplementation with $FeSO_4$ and ascorbate was still continuing (Table III).

Different oxidized DNA bases were being affected in different ways. 8-hydroxyguanine did not change at 6 weeks but had decreased by 12 weeks. 5-Hydroxyhydantoin, 5-hydroxymethylhydantoin and FAPy adenine were elevated at 6 weeks, but had normalized by 12 weeks.[67] The data could be taken to suggest that the iron/ascorbate is indeed acting as a pro-oxidant *in vivo*, increasing oxidative damage to certain DNA bases. The system then responds by up-regulating defence or up-regulating repair, or both. 8-Hydroxyguanine is one of the most efficiently repaired lesions,[37,70] so its levels are possibly unaffected, while levels of some of the base oxidation products that are repaired more slowly[37] increase and then normalize. Similar observations were made in a placebo-controlled triple supplementation study in which healthy subjects were given a mixture of vitamin E, vitamin C and β-carotene. Again, there was no

TABLE III Effects of co-supplementation of healthy human volunteers with $FeSO_4$ plus ascorbate on levels of oxidative DNA damage in white cell DNA

Base measured	Level (nmol/mg DNA) at		
	Start	6 weeks	12 weeks
8-Hydroxyguanine	0.24 ± 0.087	0.31 ± 0.081	0.12 ± 0.034 (significantly decreased)
Total base damage	2.6 ± 0.77	5.2 ± 1.90 (significantly increased)	3.1 ± 0.87

Healthy human volunteers were given 60 mg ascorbate plus 14 mg $FeSO_4$ per day. Data abstracted from Ref. [67].

change in 8-hydroxyguanine but levels of certain other DNA base damage products went up but later normalised.[71] By contrast, when we compared subjects on a flavonoid-rich diet and a flavonoid-poor diet, there were no changes in any parameters of oxidative DNA damage.

Our data are consistent with previous studies measuring 8OHdG levels in urine. Von Poppel *et al.* found that they are not decreased by giving vitamin C, vitamin E or β-carotene to healthy subjects, but that 8OHdG excretion is decreased by consuming vegetables such as Brussels sprouts.[72,73] Hence there is something in the Brussels sprout that decreases the urinary excretion of 8OHdG, and it is not β-carotene, nor vitamin C, nor vitamin E. Thus fruits and vegetables may contain compounds that decrease levels of oxidative DNA damage *in vivo* which are not the "classical" antioxidants as we know them.

One caveat about the data in[67,71] must be added: the studies were done on human volunteers. Subjects who volunteer for this kind of study are often people interested in nutrition and they eat well, so that their starting plasma levels of vitamins E, C and β-carotene are already in the range identified as optimal.[63] We are now doing studies with people who are less well-nourished, and preliminary data indicate that ascorbate/$FeSO_4$ supplementation *decreases* 8OHdG levels.[67] Perhaps healthy people, the ones most likely to take vitamin supplements are the ones that do not need them, at least in the context of oxidative DNA damage. They may even be stimulating oxidative damage to their DNA, although if that is followed by up-regulated repair it might be beneficial overall.[67]

HUMAN POLYMORPHISMS?

We have been measuring oxidative DNA damage in humans for several years now. In subjects with a comparable degree of nutrition there is still a large variation in levels of the various base damage products (Table IV and Figure 2). When examining published data from other groups, the same thing is seen (e.g. in urinary 8OHdG excretion rates and plasma isoprostane levels), although it has not been commented on until recently.[74,75] Hence some people have more oxidized DNA or lipid than others. Exploring the reasons for this (apart from the effects of nutrition) has scarcely begun.

TABLE IV Variations in levels of oxidative DNA base damage products measured in white blood cells from nineteen healthy human non-smokers

Base damage product	nmoles/mg DNA				
	Mean	SD	Median	Minimum	Maximum
5-Cl Uracil	0.06	0.02	0.06	0.03	0.10
5-OH Me Hydantoin	0.23	0.08	0.18	0.12	0.36
5-OH Hydantoin	0.12	0.04	0.12	0.06	0.20
5-OH Uracil	0.11	0.03	0.11	0.07	0.19
5-OH Me Uracil	0.03	0.01	0.03	0.01	0.05
5-OH Cytosine	0.10	0.02	0.09	0.08	0.14
Thymine glycol (cis)	0.32	0.18	0.26	0.11	0.75
FAPy Adenine	0.62	0.68	0.30	0.01	1.9
8-OH Adenine	0.37	0.22	0.28	0.14	0.93
2-OH Adenine	0.12	0.07	0.11	0.04	0.27
FAPy Guanine	0.32	0.22	0.42	0.12	0.64
8-OH Guanine	0.24	0.10	0.23	0.06	0.39
Total base damage	2.6	0.87	2.4	1.3	4.5

Data compiled by Ms. A. Rehman.

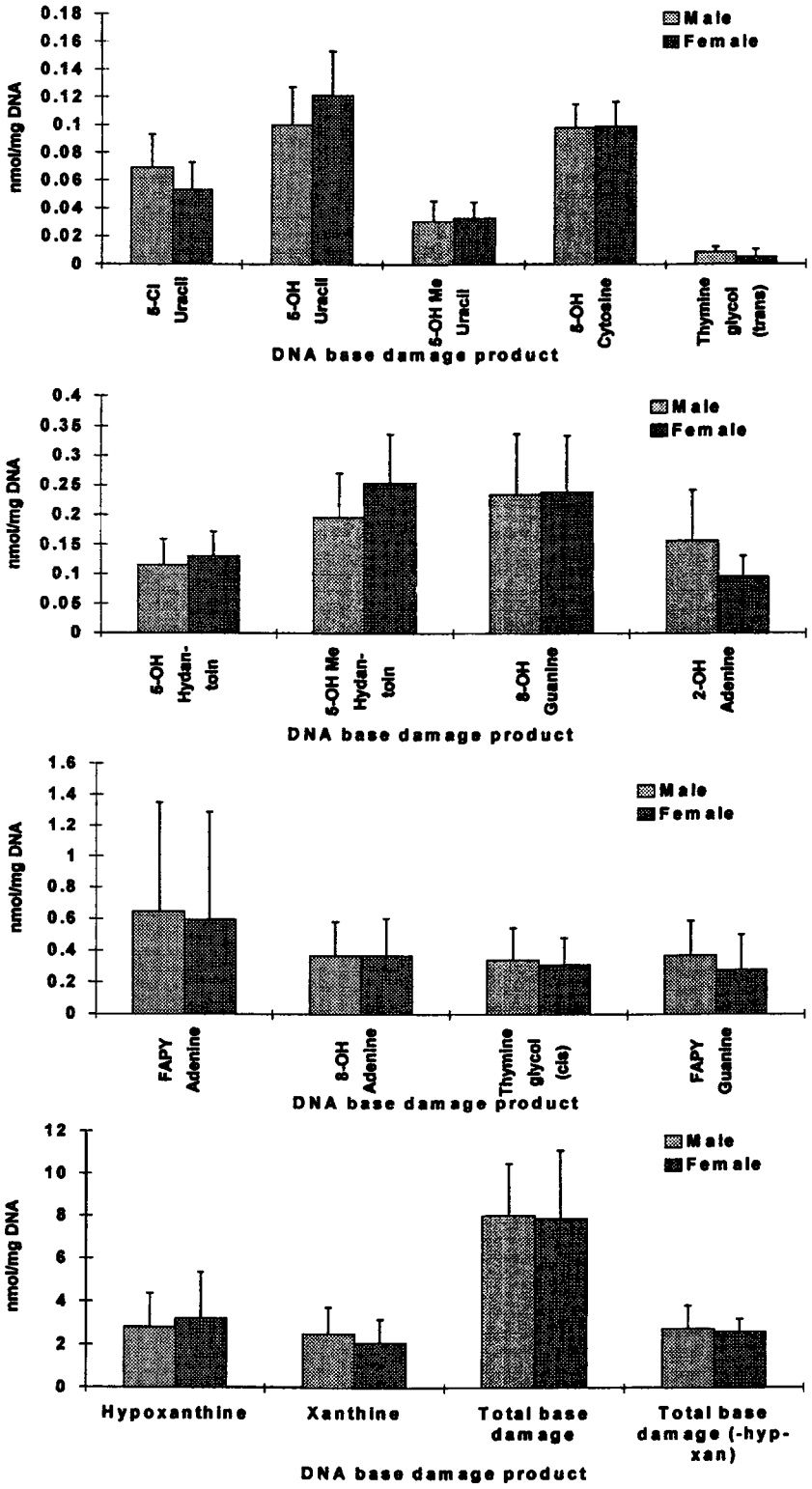

FIGURE 2 Lack of gender difference in levels of oxidative DNA damage. Total white cells were isolated from the blood of healthy male or female volunteers and analyzed for levels of oxidative DNA damage using gas chromatography/mass spectrometry. Data courtesy of Ms. Almas Rehman.

Possible Reasons?

- variations in rates of production of reactive oxygen species
- differences in the levels of antioxidant defence enzymes and other antioxidant systems
- differing efficiencies of repair of oxidative damage (especially DNA).

This area is one to which much more attention needs to be given in the future, especially as levels of oxidative damage may be predictive of the later development of cancer, cardiovascular disease or other major diseases.

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