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Review

Evolution of dietary antioxidants☆

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Abstract

Oxygen is vital for most organisms but, paradoxically, damages key biological sites. Oxygenic threat is met by antioxidants that evolved in parallel with our oxygenic atmosphere. Plants employ antioxidants to defend their structures against reactive oxygen species (ROS; oxidants) produced during photosynthesis. The human body is exposed to these same oxidants, and we have also evolved an effective antioxidant system. However, this is not infallible. ROS breach defences, oxidative damage ensues, accumulates with age, and causes a variety of pathological changes. Plant-based, antioxidant-rich foods traditionally formed the major part of the human diet, and plant-based dietary antioxidants are hypothesized to have an important role in maintaining human health. This hypothesis is logical in evolutionary terms, especially when we consider the relatively hypoxic environment in which humans may have evolved. In this paper, the human diet is discussed briefly in terms of its evolutionary development, different strategies of antioxidant defence are outlined, and evolution of dietary antioxidants is discussed from the perspectives of plant need and our current dietary requirements. Finally, possibilities in regard to dietary antioxidants, evolution, and human health are presented, and an evolutionary cost-benefit analysis is presented in relation to why we lost the ability to make ascorbic acid (vitamin C) although we retained an absolute requirement for it.

Keywords: Antioxidant; Ascorbic acid; Diet; Evolution; Human health; Oxidation; Oxidative stress; Paleolithic; Vitamin C

1. Introduction: looking back to move forward

Evolution is driven by heritable biological adaptions to a changing environment. In the evolutionary sense, adaptations are ‘traits that arise and are maintained under selection’ (Hochachka, 1998). Adaptations that improve environmental fit promote survival and reproductive success, and so become prevalent. The results of evolution are often clearly visible, however, the individual steps and pressures that led to the current evolutionary profile of life on Earth are not so easily discerned. As affirmed by Hochachka (1998), a blending of evolutionary and mechanistic physiology is needed in order for us to understand our complex phenotype and how it was shaped. Interdisciplinary study of physiological mechanisms, their control and function is needed, but we must also study their origins and history. Only by applying this integrative and extensive approach we will gain insight into our physiological limitations, recognize our evolutionary design, and know our potential as a species and as individuals. We must look back in order to move forward, using the new techniques of genomics, proteomics and metabolomics (Young, 2002) successfully to map programmed

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Table 1
Estimated daily intake of selected nutrients in Paleolithic and modern diets

Micronutrient	Paleolithic diet	Modern diet	Current day average requirement or recommended allowance
Vitamin C (mg)	604	59–115	30 (UK) 75–100 (women); 90 (men) (US)
Vitamin E (mg)	33	5.2–6.0	7 (UK); 15 (US)
Folate (μg)	357	208–317	140
Carotenoids (μg)	5560	1846–2048	Currently no recommended allowance
Iron (mg)	87	9.5–17.2	7–10
Copper (mg)	?	1–1.3	0.8
Zinc (mg)	43	7.1–13.6	5.5–7.5

Data from Eaton et al. (1997), Carr and Frei (1999), Kiely et al. (2001) and Levine et al. (2001).

interactions between genes, nutrients and environment and determine their downstream effects on human health.

Biological systems interact with the external environment to maintain an internal environment that favours survival, growth and reproduction. For all but the most fastidious anaerobes, oxygen is vital. However, the paradox of aerobic life is that oxidative damage occurs to key biological sites, threatening their structure and function. Oxygenic threat is met by an array of antioxidants that evolved in parallel with our oxygenic atmosphere. The human body uses various antioxidants, some of which are dietary-derived, for defence. The largely plant-based dietary antioxidants are believed to have an important role in the maintenance of human health because our endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species (ROS; oxidants) (Fridovich, 1998; Gracy et al., 1999; Halliwell and Gutteridge, 1999; Strain and Benzie, 1999; Benzie, 2000; McCord, 2000; Ames, 2001). In this paper, the human diet is discussed briefly in terms of its evolutionary development, and the need for antioxidants and different strategies of antioxidant defence are outlined. The evolution of dietary antioxidants is discussed from the perspectives of plant need and human requirements. Finally, possibilities in regard to dietary antioxidants, evolution and human health are presented, with particular reference to why we lost our ability to make ascorbic acid (vitamin C) and yet have retained an absolute requirement for it.

2. The evolution of the human diet

The earliest humans date from approximately 2 million years ago. However, nutritional requirements had been shaped during more than 25 million years of evolutionary development from anthropoid primates (Milton, 2000). For most of that time, macro- and micro-nutrient needs were met by a largely herbivorous diet (Milton, 1999; Wood and Brooks, 1999). It is hypothesized that the incorporation of higher quality, less fibrous food into the diet fuelled a simultaneous increase in brain development and decrease in gut size (Aiello and Wheeler, 1995; see also http://www.beyondveg.com). This more nutrient-dense hominoid diet was likely to have comprised energy rich plants (young leaves, shoots, roots and fruits) and regular amounts of animal protein and fat (Milton and Jenness, 1987; Milton, 1993; Aiello and Wheeler, 1995; Eaton et al., 1997; Popovich et al., 1997; Wood and Brooks, 1999; Milton, 1999, 2000). The exact components of the early human 'hunter–gather' diet are not known, however, Paleolithic nutrient intakes have been estimated by Eaton et al. (1997). These are far from those of the energy-dense, highly digestible, micronutrient-poor diet of most modern day humans. In particular, the Paleolithic intake of plant-derived antioxidants is considered to have been many times higher than current intake (Table 1).

Organised agriculture began approximately 12 000 years ago, and stimulated a pace of dietary and social changes that far outstripped our biological ability to adapt. This has led to the hypothesis that the various and common 'diseases of civilization' are rooted in a chronic mis-match between our ancient (but still current) nutritional programming and our contemporary dietary input (Boyd Eaton et al., 1988; Milton, 1999), a mis-match that has been revealed owing to the relatively recent increase in average lifespan that has resulted from marked improvements in living conditions

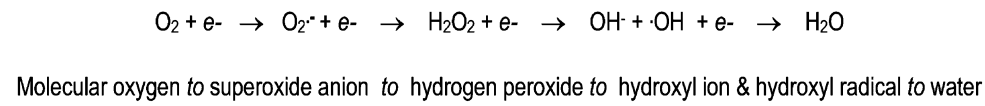

Fig. 1. Simplified representation of single electron reductions of molecular oxygen biradical.

and healthcare provision. A key mis-match between dietary supply and physiological need may be in antioxidant micronutrients (Pauling, 1970; Gey, 1998; Lampe, 1999; Strain and Benzie, 1999; Ames, 2001; Block et al., 2001). This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases, and that dietary antioxidants oppose this and lower risk of disease (Ames et al., 1993; Beckman and Ames, 1998; Fridovich, 1998; Gey, 1998; Benzie, 1999a; Carr and Frei, 1999; Gaziano, 1999; Halliwell and Gutteridge, 1999; Chisholm and Steinberg, 2000; Finkel and Holbrook, 2000; McCord, 2000; Khaw et al., 2001). However, while there is convincing evidence of a role for antioxidant-rich diets in the promotion of successful ageing and functional longevity, it is not established which, if any, specific antioxidants are responsible. A consideration of our origins and the biological need of plants and animals, including ourselves, for antioxidant defence may be helpful in understanding the possible rationale for antioxidants in health promotion.

3. Antioxidants: why they are needed and how they work

The external environment on Earth has changed dramatically since life began over 3 billion years ago (Cloud, 1968; Graham et al., 1995; Berner, 1997). The early hydrogen-rich atmosphere became increasingly oxygenic as a result of the photosynthetic activity of blue–green algae (Broda, 1975). Atmospheric oxygen content is estimated to have peaked at approximately 35% some 300 million years ago, dropped to 15% in the late Paleozoic and then reached the current day level of 21% approximately 150 million years ago (Graham et al., 1995). The biological threat imposed by increased contact with oxygen was turned to opportunity in the form of highly efficient aerobic pathways of catabolism (Barnabas et al., 1982; Barja, 1993; Berner, 1997; Benzie, 2000). However, the threat of oxygen toxicity remained. Key questions are: why is oxygen toxic? and, how do biological systems defend against it?

A ‘free radical’ is capable of independent existence and has an unpaired electron in an orbital (Fridovich, 1998; Halliwell and Gutteridge, 1999). The presence of an unpaired electron increases reactivity, as the solitary electron seeks a partner for stability. A partner can be obtained by abstracting an electron from a co-reactant. This reaction results in quenching by reduction (electron addition) of the radical and formation of a new radical by oxidation (electron loss) of the co-reactant. Oxygen is a biradical, and four electrons (two per atom) are needed (along with hydrogen) for the complete reduction of molecular oxygen to water. However, there is a large kinetic barrier to this reaction because the unpaired electrons in an oxygen atom are in parallel spins. Paired electrons (occupying the same orbital) must spin in opposite directions. ‘Spin-fitting’ of abstracted electrons does not occur spontaneously, and complete reduction by the simultaneous addition of four electrons to a molecule of oxygen is limited largely to the cytochrome oxidase-controlled final step in the mitochondrial electron transport chain. However, single-electron reduction of oxygen can occur, and this forms partially reduced oxygen intermediates (‘oxygen free radicals’; oxidants; ROS). These are potentially problematic because they are more reactive than ground state molecular oxygen (Fridovich, 1998; Gracy et al., 1999; Halliwell and Gutteridge, 1999; McCord, 2000) (Fig. 1).

ROS are formed within the body by various physiological processes and insults (Fig. 2). The anion superoxide ($O_2^{\bullet -}$) is a key oxidant because it is produced constantly and unavoidably in the mitochondria from electron leakage during their passage along the respiratory chain. Furthermore, superoxide is the source of several other ROS, including the readily diffusible hydrogen peroxide and the very reactive peroxynitrite and hydroxyl radical (Halliwell and Gutteridge, 1999; Benzie, 2000; McCord, 2000). Generation of superoxide is increased greatly during exercise and post-ischaemic reperfusion, and in inflammation. In

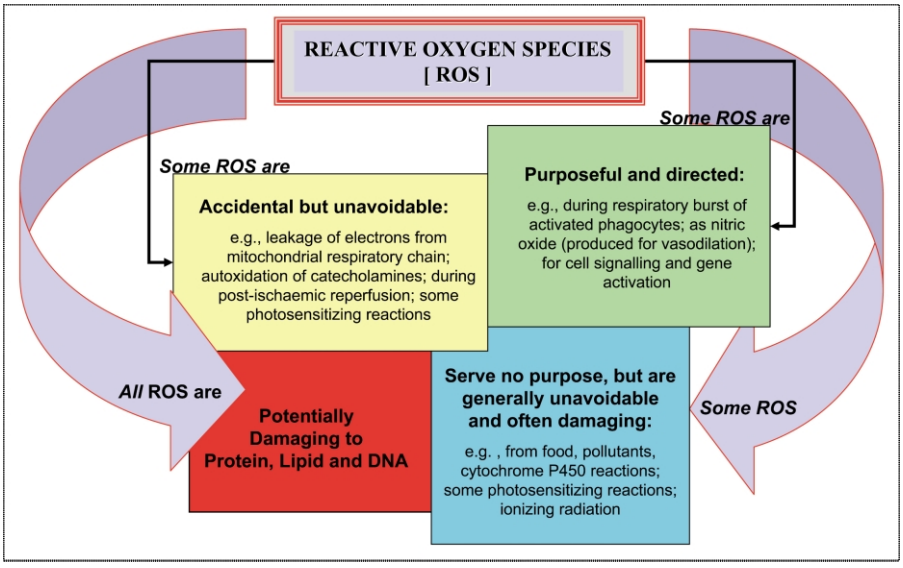

Fig. 2. Sources and characteristics of ROS.

addition, oxygen can be split by high-energy ionizing radiation, forming the fiercely reactive hydroxyl radical, and oxygen can absorb electromagnetic radiation, forming an 'energized' molecule (singlet oxygen) that has an energy level 92 kJ/mol or more above ground state molecular oxygen (Fridovich, 1974). Singlet oxygen formation occurs when photosensitisers, such as flavin-containing compounds, porphyrins and chlorophyll, are illuminated in the presence of oxygen (Halliwell and Gutteridge, 1999; Choy et al., 2000). Sites of intense oxidant challenge in the human body, therefore, include the mitochondria, the eye, the skin, areas of inflammation or post-ischaemic reperfusion. In plants, the light harvesting, photosynthesizing, oxygen-generating sites are exposed to a particularly high oxidant load.

The survival times of ROS in biological systems relate inversely to their reactivity (Halliwell and Gutteridge, 1999). Release of energy from singlet oxygen returns this ROS to ground state, but can result in damage to adjacent molecules, such as amino acids and unsaturated fatty acids. Superoxide is generally accepted to be an oxidant, but it can act as a reducing agent by donating its additional electron to an oxidized transition metal ion [Fe(III) or Cu(II)]. Superoxide is an anion and unable to move far from its site of formation owing to lipophilic membrane barriers, however, the enzyme superoxide dismutase (SOD) transforms superoxide into hydrogen peroxide, which is small, uncharged, and diffuses readily within and between cells. Hydrogen peroxide is not highly reactive and may accumulate. Concentrations of 100 μmol/l or more have been detected in biological fluids (Halliwell et al., 2000). This relatively large amount may reflect ex vivo or post-production changes in these fluids rather than the true in vivo levels, but significant amounts are likely to be present in vivo, and these represent a potential threat to biological systems because hydrogen peroxide reacts with reduced metal ions in the Haber–Weiss reaction (named the Fenton reaction when the metal species is iron), forming the hydroxyl radical. This ROS is highly reactive, and interacts with the first thing it contacts, causing rapid and indiscriminate damage to biological substrates (Fridovich, 1974; Halliwell and Gutteridge, 1999; McCord, 2000).

Clearly, a mixture of superoxide, its product hydrogen peroxide, and transition metal ions is a combination best avoided. Superoxide also reacts with nitric oxide ($NO^{\bullet}$), an important vasodilator, to form the very reactive peroxynitrite species ($HNOO^{-}$), and superoxide generated within activated phagocytes forms hypochlorous acid (HOCl), which aids in bacterial killing (Halliwell and Gutteridge, 1999). Generation of various ROS, therefore, stems from superoxide, and this cannot

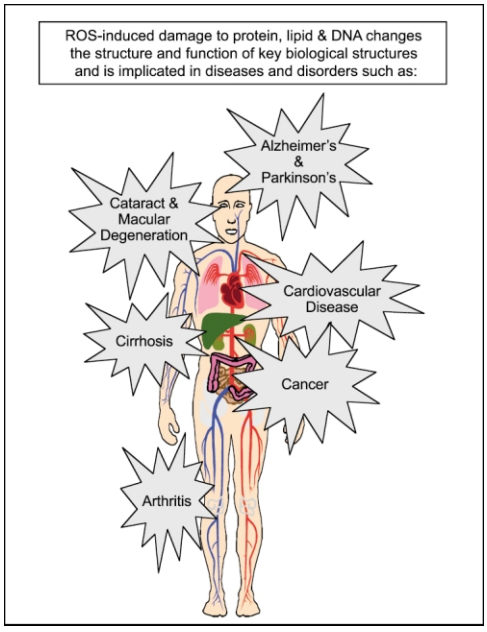

Fig. 3. ROS and disease.

be totally prevented at any point in the chain of formation. Indeed, even if this were possible, it is becoming increasingly clear that it is not desirable, as many ROS have important roles in physiological processes and systems, including microbial killing, blood pressure control, endothelial function, cell signalling, apoptosis, cell division, and gene transcription (Suzuki et al., 1997; Dalton et al., 1999; Halliwell and Gutteridge, 1999; Clement and Pervaiz, 2001). Metal ions cannot be entirely eliminated, as they are an intrinsic part of crucial biological macromolecules, including haemoglobin, caeruloplasmin and SOD. 'Anti-oxidation' strategies are needed, therefore, to limit damaging interaction between ROS and valuable substrates such as protein thiol groups, DNA bases, and polyunsaturated fatty acids (PUFA). Oxidative damage to these macromolecules can lead to enzyme inactivation, mutation, membrane disruption, increased atherogenicity of low density lipoproteins, mitochondrial dysfunction, and cell death. These are the toxic effects of oxygen, and they are implicated in ageing and in the development of chronic, inflammatory, degenerative, and age-related diseases (Beckman and Ames, 1998; Halliwell and Gutteridge, 1999; Finkel and Holbrook, 2000; McCord, 2000) (Fig. 3).

To deal with the threat of oxidant-induced damage, biological antioxidants evolved (Fridovich, 1998; Benzie, 2000). An antioxidant can be defined in simple terms as anything that inhibits or prevents oxidation of a susceptible substrate. Antioxidant systems are complex, however, and act in concert to decrease ROS load, to divert ROS to other reaction pathways with less reactive products, to selectively inactivate (in redox terms) transition metal ions, and, when all else fails, to provide sacrificial molecules that act as a replaceable or recyclable 'buffer' to absorb oxidative hits and excess energy (Table 2).

Human antioxidant defences are effective but they are not infallible, and oxidative damage to key biological sites occurs, accumulates with age, and contributes to senescence and age-related disease (Ames et al., 1993; Beckman and Ames, 1998; Halliwell and Gutteridge, 1999; Finkel and Holbrook, 2000). This is not, however, the failure of evolution it may at first seem. Rather, the shortfall in our antioxidant defence is based on a fundamental evolutionary principle. Adaptive changes are selected for on the basis of physiolog-

Table 2
Mechanisms of antioxidant action

Physical barriers prevent ROS generation or ROS access to important biological sites, e.g., UV filters, cell membranes
Chemical traps/sinks 'absorb' energy and electrons, quenching ROS, e.g., carotenoids, anthocyanidins
Catalytic systems neutralise or divert ROS, e.g., the antioxidant enzymes SOD, catalase and glutathione peroxidase
Binding/inactivation of metal ions prevents generation of ROS by Haber–Weiss reaction e.g., ferritin, caeruloplasmin, catechins
Sacrificial and chain breaking antioxidants scavenge and destroy ROS, e.g., ascorbic acid (vitamin C), tocopherols (vitamin E), uric acid, glutathione, flavonoids

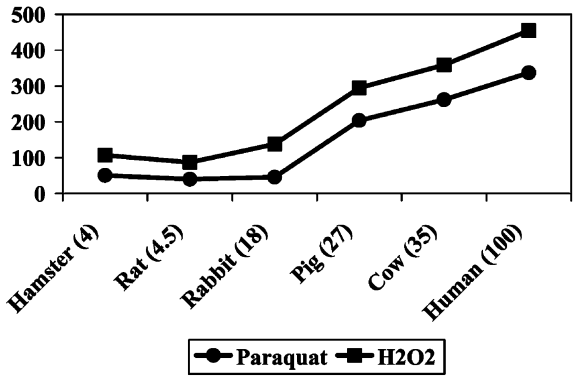

Fig. 4. Positive correlation between mammalian lifespan and cellular resistance to oxidative stress induced by paraquat and hydrogen peroxide in species with different lifespans (years) [Data from Kaphi et al., 1999].

ical (reproductive) benefit in relation to metabolic cost. Consequently, the evolution of our endogenous antioxidant system has not progressed beyond the 'break-even' point of cost effectiveness. This means that oxidative stress, which is an oxidant:antioxidant imbalance in favour of oxidation, remains a real and constant threat.

Conceptually, if oxidative damage leads to ageing, dysfunction and disease, then preventing it will delay ageing and help prevent disease (Beckman and Ames, 1998). This concept underlies the hypothesis that increased antioxidant defence benefits health and promotes functional longevity. Interestingly, it has been noted (Cutler, 1984) that the maximum lifespan potential (MLSP) of different species correlates directly with their plasma levels of uric acid, an endogenous antioxidant, when these are corrected for metabolic rate, and mammalian lifespans have also been reported to correlate directly with resistance of cellular structures to oxidative challenge (Kapahi et al., 1999) (Fig. 4).

The question then arises, can antioxidant defence be enhanced to prevent oxidative stress? Increased melanin in skin in response to exposure to ultraviolet light reflects increased local defence, and it has been suggested (Cutler, 1984) that the increases seen in plasma uric acid with age and in certain disorders indicates systemic upregulation of antioxidant synthesis in response to increased need. Furthermore, glutathione (GSH) and antioxidant enzymes increase in response to regular increases in ROS load caused, for example, by exercise (Clarkson and Thompson, 2000). These changes in endogenous antioxidants may reflect a physiological attempt to restore balance in the face of established oxidative stress. A more pre-emptive and immediate strategy, however, may be to increase intake of dietary antioxidants, thereby filling the evolutionary gap between an acceptable and an optimal antioxidant defence (Gey, 1998; McCall and Frei, 1999; Ames 2001).

4. Dietary antioxidants: from plant need to human requirements

The photosynthetic site and its immediate surroundings are exposed to high levels of oxygen, and singlet oxygen is formed by transfer of photons of energy from chlorophyll, a photosensitiser, within the chloroplast (Halliwell and Gutteridge, 1999). For early photosynthetic plants, oxygen was a toxic waste product, and strategies to protect against oxygen and its more reactive forms evolved (Fridovich, 1974; Benzie, 2000). Defences became more sophisticated and specific as need increased, and progressed to catalytic systems to divert superoxide to the less reactive (but still potentially problematical) hydrogen peroxide, and to transform this ROS into water. Photosynthetic plants also produce quenchers of singlet oxygen, resonant 'absorbers' of single electrons, and compounds that bind metal ions, scavenge ROS, and break chains of oxidation. Plants, therefore, produce a very impressive array of antioxidant compounds that includes carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols (Strain and Benzie, 1999; Hollman, 2001). These are concentrated in the oxidation-prone sites of the plant. For example, up to 50 mM concentrations of ascorbic acid (vitamin C) are found in chloroplasts; this is approximately 1000 times higher than the ascorbic acid concentration of human plasma (Davey et al., 2000). The lipophilic carotenoids, which include lutein, violaxanthin and β carotene, are light harvesters that transfer energy to chlorophyll, but are also important in defence against photo-oxidative damage to lipid-rich components (van den Berg et al., 2000). Oils in leaves and seeds are rich in tocopherols and tocotrienols ('vitamin E') (Bramley et al., 2000). Some plant-derived compounds with antioxidant properties can serve other defensive purposes, however. Tannins, which are water-soluble phenolic compounds that can precipitate proteins, have a bitter taste and impede absorption

of plant protein. Unripe seeds are high in tannins, and this discourages herbivores from eating seeds before they are ripe and ready for dispersal (Mehansho et al., 1987). Tannins also increase the resistance of cereal grains to fungal moulds, and can be toxic to insects. Terpenes, which are plant-based derivatives of lanosterone, are also toxic to insects. Ascorbic acid synthesis in plants increases in response to wounding or harsh environmental conditions, and during growth and fruit ripening (Davey et al., 2000). Phenolic acids have antibacterial and antiviral activity, and may act as plant signalling compounds (Parr and Bolwell, 2000). Plant antioxidants, therefore, act in various ways to promote plant survival and reproduction.

Many plants and flowers are brilliantly coloured in shades of red, orange, yellow, and purple. The colours are bestowed by a huge range of anthocyanidins, a class of flavonoids, and carotenoids (Jackson et al., 1996; Lindsay and Clifford, 2000). These compounds are singlet oxygen quenchers and UV absorbers, but their colours attract birds, insects and animals that act as vectors for pollen and seed transfer. Plants clearly benefit from this arrangement, but so, presumably, do those animals that have developed a visual system that can distinguish colours. Interestingly, most carnivores, which do not rely directly on plants for antioxidants, see colours very poorly, while fruit-eating insects, birds, humans and other primates have well developed colour vision (http://lsvl.la.asu.edu/askabiologist/research/seecolour). Carnivores obtain vitamin E from body fat and lipid-rich tissues, and additional vitamin C from organ meats. Organ meats and fat are eaten in preference to muscle meat, and liver and kidney are the sites of ascorbic acid synthesis in, respectively, mammals and reptiles (Chatterjee, 1973), while the adrenals and eyes are very rich in vitamin C.

Plants that need cross-pollination or seed transfer, therefore, have evolved to make use of brightly coloured antioxidant compounds to signal their presence. Carnivorous animals that are not directly dependent upon plants as a source of antioxidants presumably did not experience selection pressure favouring colour vision, but animals that need these brightly coloured antioxidant compounds, or something closely associated with them, did because it helped in identifying their dietary supply. Interestingly, the maculae of eyes that see colour are rich also in yellow/orange carotenoids (Halliwell and Gutteridge, 1999; Choy et al., 2000). Just as they are designed to do in plants, these diet-derived quenching antioxidants protect animal sites against photo-oxidative damage, and are concentrated at the site of special need, the light exposed, photosensitised, PUFA-rich retina. The antioxidant vitamins C and E also protect our protein, lipid and DNA, presumably in the same way that they protect those of plants, because the types of ROS generated within plants and animals are the same. But why is it that we are so dependent upon dietary antioxidants, rather than meeting our needs by endogenous production?

5. Human requirements for dietary antioxidants—an evolutionary mistake?

Within the human body, a variety of different antioxidants forms a co-ordinated, organism-wide system of defence (Halliwell and Gutteridge, 1999; Benzie, 2000), although whether this system has an overall control mechanism is not yet clear. Some antioxidants, such as the enzymes SOD and GPx, react with a specific type of ROS, while others are less discerning and act as effective scavengers. Just as different body compartments use different buffering systems to maintain pH, so the antioxidant profiles of different body sites are different (Halliwell and Gutteridge, 1999; Choy et al., 2000; Polidori et al., 2001). The variety of endogenous antioxidants in human biological fluids is impressive, but incomplete, and dietary input of plant-based antioxidants, most notably the antioxidant vitamins C and E, is needed (Gey, 1998; Strain and Benzie, 1999). It is hypothesized that not only do we need to prevent overt deficiency of these, and perhaps other dietary antioxidants, but that health benefits continue to increase with the amount taken. This hypothesis is based on the undisputed health benefits of diets rich in fresh fruits and vegetables (Benzie, 1999b; Lampe 1999; Hollman, 2001; Block et al., 2001). Epidemiological evidence of protection against disease is so strong that five or more servings of fruits and vegetables daily are recommended, and an increase of one serving daily has been estimated to decrease cancer risk by 14% (World Cancer Research Fund and the American Institute for Cancer Research, 1997; Khaw et al., 2001). To date, research on antioxidant micronutrients has focused largely on α-tocopherol and ascorbic acid (Bramley et al., 2000; Davey et al., 2000). However, carotenoids,

flavonoids and other plant antioxidants may also be important for human health, although no dietary requirement for these has yet been identified (Table 3). While it cannot be ruled out that dietary antioxidants are mere co-travellers with as yet unidentified beneficial agent(s), and may in some circumstances act as pro-oxidants or have toxic effects, there is, nonetheless, convincing evidence that increased intake of dietary antioxidants promotes human health (Benzie, 1999a; Carr and Frei, 1999; Ames, 2001; Block et al., 2001; Hollman, 2001).

However, why is it we need any dietary antioxidant input at all? The answer is likely to be because it is easier to eat something than to make it de novo. If dietary antioxidant supplies were adequate, no selection pressure would have existed for endogenous production to evolve. This is likely to be the case for most plant-based antioxidants, including vitamin E and folic acid and, possibly, carotenoids and flavonoids. However, the scenario for ascorbic acid is different. Humans are one of the few vertebrates that cannot make vitamin C, although we are descended from an ancestor that could. At some point in our evolutionary development, therefore, we lost the ability to make ascorbic acid, even though an absolute need for it was retained.

Humans, non-human primates and a very few other mammals cannot make ascorbic acid because the gene for the flavo-enzyme, L-gulono-1,4-lactone oxidase (EC1.1.3.8) is not transcribed. This enzyme catalyses the final step in ascorbic acid synthesis and, although we retain the gene, it is highly mutated (Chatterjee, 1973). Deficiency of ascorbic acid (scurvy) was described in ancient times, and although James Lind demonstrated in the mid-18th century that the disease could be prevented by intake of citrus fruits, it was less than 100 years ago that scurvy was fully accepted to be caused by a dietary deficiency, and it was just over 70 years ago that ascorbic acid was identified as 'vitamin C' (see review by Davey et al., 2000). Scurvy can be prevented by a daily intake of only 10 mg vitamin C, but it is believed that markedly higher intakes are needed for optimal health, perhaps stemming from our ancient dietary habits (Pauling, 1970; Boyd Eaton et al., 1988; Gey, 1998; Carr and Frei, 1999; Ames, 2001; Levine et al., 2001). In response to evidence supportive of benefit, the US recommended daily reference intake has been increased recently to 75 mg for women and 90 mg for men (Food and Nutrition Board, 2000), however, this is only a fraction of the >2 g daily requirement proposed by Pauling (1970), and is several times lower than that estimated to have been ingested daily by our Paleolithic ancestors (Eaton et al., 1997) (Table 1). Interestingly, human tissues reach saturation point with ascorbic acid intakes of <200 mg/day, and relatively less ascorbic acid is absorbed as the ingested dose is increased (Davey et al., 2000). Therefore, while it is recognized that intake above that needed to prevent simple deficiency is beneficial, the direct benefits of very high intakes of the vitamin, as opposed to moderate intake, are unclear. Whether Paleolithic requirements were greater per se, or daily intakes far exceeded need is not known, and what human optimal requirements are under various environmental and physiological conditions is still debated. Nonetheless, it is clear that we have an absolute dietary requirement for ascorbic acid, and the loss of our ability to make this vital substance seems paradoxical.

Several theories have been suggested to account for this apparently counter-productive adaptation. These include loss of ascorbic acid as a means of population control (Millar, 1992), protection against malaria (Calabrese, 1982), and increasing mutation-driven evolution of Homo Sapiens (Challem, 1997). These suggestions are not convincing. Cutler (1984) argued that our ability to produce the enzyme uricase was lost at around the same time as our ability to produce L-gulono-1,4-lactone oxidase, effectively resulting in replacement of ascorbic acid by uric acid as the major endogenous water-soluble scavenging antioxidant in human biological fluids (Cutler, 1984). Human uric acid levels are relatively high, and uric acid is a scavenging antioxidant (Ames et al., 1993). However, high plasma levels of uric acid are not beneficial to health (Benzie and Strain, 1996). Furthermore, the presence of uric acid has not eliminated our need for ascorbic acid. Therefore, while their actions may be complementary or co-operative, it is clear that ascorbic acid was not replaced by uric acid in human antioxidant defence.

Was loss of ascorbic acid synthesis an evolutionary mistake? This is unlikely. Logically, the loss of endogenous production of ascorbic acid must have conferred survival and reproductive benefit for it to have endured and prevailed during our evolutionary development, and the mechanism

Table 3
Dietary antioxidants: source, bioavailability and concentrations in human plasma

	Source	Bioavailability	Concentration	Comment
Ascorbic acid (vitamin C)	Fruits and vegetables, particularly strawberries, citrus, kiwi, Brussels sprouts, cauliflower, some Chinese green vegetables	100% at low doses (<100 mg) decreasing to <15% at >10 g	25–80 μM	Unstable at neutral pH; concentrated in cells and the eye
'Vitamin E' (α, β, δ, γ isomers of tocopherols and tocotrienols)	Green leafy vegetables, e.g., spinach; nuts, seeds, especially wheat germ; vegetable oils, especially sunflower	10–95%; hepatic uptake saturable process	15–40 μM	Major tocopherol in diet is γ form, but α form is preferentially taken up by human liver
Carotenoids (hundreds)	Orange/red fruits and vegetables (carrot, tomato, apricot, melon, yam; green leafy vegetables, broccoli)	Unclear; dose and form dependent; probably <15%	Very low (<1 μM)	Lutein and zeaxanthin are concentrated in macula region of the eye
Flavonoids (enormous range of different types)	Berries, apples, onions, tea, red wine, some herbs (parsley,thyme) citrus fruits, grapes, cherries	Most poorly absorbed; depends on form and dose; quercetin absorption 20–50%; catechins <2%	No data for most; probably total never >3 μM	Quercetin and catechins may be most relevant to human health as intake is relatively high and there is some absorption

Data from Jackson et al. (1996), Lindsay and Clifford (2000) and Szeto et al. (2002) and other sources.

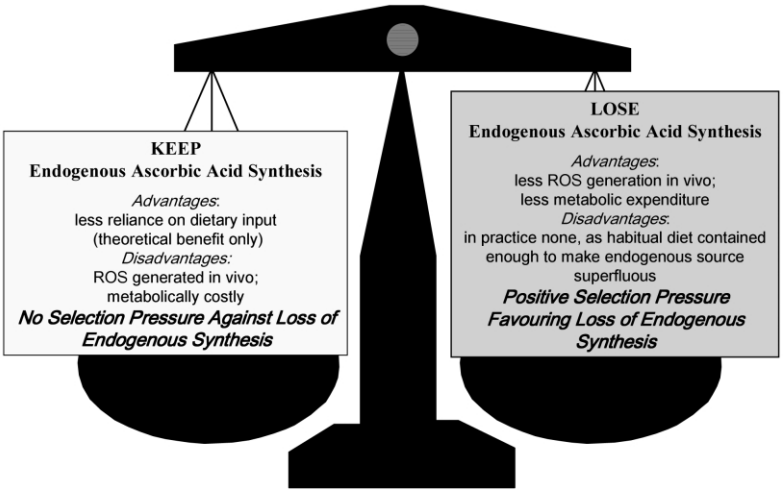

Fig. 5. Loss of ability to synthesize ascorbic acid—simple evolutionary economics?

of benefit may have been quite simply economics. ROS are produced during ascorbic acid synthesis, and preventing this may have been an effective strategy to decrease ROS load. Provided, dietary intake was high enough to make up for the lack of endogenous ascorbic acid, this strategy would decrease ROS load and produce a metabolic saving (Pauling, 1970; Benzie, 2000). It is worth noting here that intake of iron was very high in the Paleolithic diet (Table 1) and, as redox active iron and ascorbic acid is a potentially dangerous mixture, it is possible that loss of endogenous synthesis of ascorbic acid conferred additional benefit by limiting the potential for Fenton chemistry. However, as the daily intake of ascorbic acid in the largely herbivorous early hominid diet was approximately 10 times current intakes (Eaton et al., 1997), it is unlikely that loss of endogenous ascorbic acid would have had much effect on ROS produced from iron/ascorbic acid interaction. It can be argued, however, that an endogenous source of the vitamin was both superfluous and metabolically expensive and that we are likely to have lost the ability to synthesize ascorbic acid because it was metabolically more cost-effective to obtain it in the habitual, plant-rich diet (Fig. 5).

The issue of iron is an interesting one though, as high intake may induce a potential, additional oxidative stress in consumers (Halliwell and Gutteridge, 1999). Many plant-based antioxidants bind iron, however, as well as scavenging ROS. More importantly perhaps, the type of iron in the diet must be considered. In the Paleolithic diet this was likely to have been largely non-haem iron. The absorption of non-haem iron is affected by other dietary components. Absorption is increased by ascorbic acid, while legumes and polyphenolic components of plant-based food can virtually block iron absorption (Frossard et al., 2000). The inhibitory effect of various dietary constituents may have prevented excessive absorption of iron, therefore, even though Paleolithic intake was high. Alternatively, the concurrent high antioxidant intake may have offset the oxidative potential of dietary iron. In the modern diet, however, haem iron is more plentiful. This is generally absorbed well, and high intake of haem iron can lead to overload. Antioxidant requirements in relation to differential iron intake in the modern diet, therefore, is an area worthy of study.

A final consideration of our dietary antioxidant requirements is in relation to the environment in which our ancestors evolved. Not only was their intake of plant-based antioxidants higher, but also the demand may have been less. Hominids evolved in the increasingly hypobaric hypoxic conditions of the Rift Valley, and this may have favoured a highly efficient aerobic ancestral phenotype (Hochachka, 1998), which evolved (to the break-even point) to suit the prevailing environment. Thereafter, some of the ancient evolutionary adaptations may have been retained, upregulated or selected against, depending upon the changed environment in which different human lineages devel-

oped over the ensuing 100 000 or so years (Hochachka, 1998). However, enhancing antioxidant defence through increased dietary intake (if a beneficial strategy) removes selection pressure for remodelling endogenous antioxidant systems to improve defence, and could even result in a blunting or downregulating of endogenous antioxidants (as is suggested for ascorbic acid). It is likely also that there has not been enough time for phylogenetic adaptation to the relatively hyperbaric, more oxygenic environment in which most humans now live. It could be argued, therefore, that humans who do not live in the high, dry, relatively hypoxic environment of their reported origin are out of step with the surroundings that their ancient physiological phenotype is designed to deal with. If so, these groups may benefit most from increased intake of dietary antioxidants. It would be interesting to compare biomarkers (Halliwell, 1999) of antioxidant status and oxidative stress in the different human lineages that have lived in different oxygenic environments in recent history, and to determine their responses to increased supply of dietary antioxidants. Such a study would help answer the questions of whether plant-based antioxidants can and do complement and augment the action of human endogenous antioxidants in opposing oxidative stress, and whether increased dietary antioxidants can promote functional longevity.

6. Evolution of dietary antioxidants in the context of human health and dietary needs: an evolution-based hypothesis, and the need for an interdisciplinary research agenda

In this era of genomics, proteomics and metabolomics (Young, 2002), the challenge in nutritional science is to clarify human nutrient requirements and design nutritional strategies to promote health and functional longevity. This requires an interdisciplinary approach that, as stated by Young (2002) 'is the key to the future and the vigorous evolution of nutritional science'. Wood and Brooks (1999) suggested that 'we are what we ate' and, while many geographical and environmental factors undoubtedly determined the evolutionary development of our species, our future health as individuals may well depend upon what we eat today. We have various endogenous antioxidants, but they are inadequate to prevent oxidative damage, and increased intake of dietary antioxidants may help maintain the balance between oxidants and antioxidants, slow age-related changes, and delay the onset of age-related disease. High intake of plant-based food indisputably benefits human health, however, it is not yet known whether it is the antioxidant constituents that are responsible, whether the benefit is of a threshold type or on a continuum, or if it is one, many or all plant-based antioxidants that are involved. Furthermore, it cannot be entirely discounted that antioxidants are mere co-incidental dietary co-travellers with other, as yet unknown, beneficial ingredient(s) or effects of plant foods.

To an extent we can say that evolution supplies the answers, and all we have to do is to find the right questions. Therefore, with the large body of evidence that increased intake of dietary antioxidants benefits human health, a rewarding approach may be to ask why our endogenous antioxidant defence is inadequate and needs to be supplemented by dietary antioxidants? Why do we not have some endogenous antioxidant 'reserve' to prevent, and not just oppose, oxidative damage? The evolutionary answers may lie in the cost-benefit limitations on heritable adaptations and in the environmental conditions of early hominid evolution. Evolutionary strategy is simple economics. When the break-even point is reached in a physiological adaptation process, no further resources will be spent in upregulating the adaptation. Consequently, any small physiological advantage conferred by upregulating endogenous antioxidant defences beyond our current levels was clearly not worth the additional metabolic cost, and indeed there must have been a benefit during our evolution to the loss of endogenous synthesis of ascorbic acid. The economy-driven strategy is particularly true if the benefit of additional metabolic cost is seen largely in the post-reproductive years and when extrinsic mortality is high. Our traditional diet was high in plant-based antioxidants, and it is possible to speculate that this, in association with the relatively hypoxic environment in which humans evolved, resulted in endogenous synthesis of ascorbic acid becoming an expensive and superfluous process. We can hypothesize also that our early diet produced an optimal physiological oxidant/antioxidant balance that promoted survival and reproduction, and that this optimal balance is nowadays rarely achieved.

The implications for modern day dietary requirements are far-reaching and important. In our

modern world, where we stand on the continuum between health and disease and how much of our MLSP we achieve is no longer a result of a hazardous external environment. Mortality is due mainly to intrinsic processes that result in chronic, degenerative diseases such as cancer, cardiovascular disease and dementia. Because of high extrinsic mortality, the lifespans of our ancestors were short, and we do not know if their antioxidant-rich diet could have promoted health in later life. However, there is no evidence that intake of antioxidant-rich foods in early- to mid-life has any detrimental effects in later life. Indeed, there is strong and convincing evidence for the opposite being true, and that long-term intake of antioxidant-rich foods promotes healthy ageing and functional longevity. We can speculate, therefore, that the augmentation of antioxidant defence by dietary means is physiologically beneficial at all stages of life. Marked improvements in basic nutrition, sanitation, shelter and, latterly, healthcare provision have been the primary causes of the dramatic increases in life expectancy seen since Paleolithic times. Perhaps by regaining our ancestral optimal antioxidant:oxidant balance marked improvements in functional longevity can be achieved.

There are many interdisciplinary issues relating to this hypothesis that need further study, however. These include determining the bioavailability of many plant antioxidants, influence of iron status and relatively hypo- or hyper-oxygenic environments on endogenous antioxidant defences and oxidative stress, differences between different human lineages, molecular interactions between specific antioxidants, genes, biochemical pathways and organelles, and their functional outcome in terms of physiological and pathological endpoints (Benzie, 1999b).

In conclusion, plants harvest and supply the energy all other living things need. Plants produce the oxygen that is both vital for life and damaging to tissues. Early plants evolved an array of antioxidants to defend their own structures against oxidants produced during photosynthetic light harvesting and oxygen production. The human body must defend itself against the same types of oxidants, and we have also evolved an antioxidant system. This is effective, but not infallible. Reactive oxidant species breach the defences, oxidative damage ensues, and this accumulates with age, leading to a variety of pathological and ultimately fatal changes. Plant-based, antioxidant-rich foods traditionally formed the major part of the human diet. This may have led to high antioxidant status at low metabolic cost, with subsequent cost-effective loss of ascorbic acid synthesis. In addition, high dietary intake of antioxidants may well have relieved the pressure for further evolutionary development of endogenous defences. The hypothesis that higher intake of plant foods is beneficial to human health because this increases antioxidant defence against oxidative damage is logical in evolutionary terms, especially when we consider the relatively hypoxic environment in which humans may have evolved. However, it will remain a mere hypothesis unless validated scientifically by interdisciplinary research that considers not only what we are, but also how and why we became so.

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