

The potential for interventional use of antioxidants in clinical disease

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KEY POINTS

- Endogenous oxidative damage to cellular components, primarily proteins, lipids and DNA, is thought to be a contributory factor in the pathogenesis of numerous chronic diseases
- Natural antioxidants, such as vitamin E and taurine, function to protect membrane and cytosolic components against free radical damage and act as the body's major defence against oxidative stress.
- In humans, there is a well-documented link between compromised antioxidant status, indices of oxidative damage and clinical disease.
- Potential roles for antioxidant therapy have been identified in a range of human diseases including diabetes mellitus, asthma, chronic renal failure, hepatitis, colitis, atopic dermatitis and arthritis.
- There is a strong potential to attenuate oxidative stress in these disorders via dietary manipulation in canine and feline therapeutic diets.

The association between compromised antioxidant status, indices of oxidative damage and clinical conditions including diabetes mellitus, asthma, chronic renal failure, hepatitis, colitis, atopic dermatitis and arthritis is now well documented. However, the extent to which oxidative stress plays a causal role in the onset or progression of disease is unclear. There is considerable circumstantial evidence linking diminished antioxidant status (including enzymes and non-enzymatic scavengers) to increased oxidative damage and disease severity. However, there have been few dietary intervention studies, and of those that have been reported, the results have been equivocal. Meanwhile there is no doubt that the increased demand on non-enzymatic defences can precipitate nutrient deficiency in physiologically stressed subjects. This has particular relevance in the context of cats and dogs maintained long term on diets formulated to meet the minimum nutritional requirements as defined in studies using healthy animals.

This review explores the evidence surrounding two nutritional

antioxidants: vitamin E and taurine. It focuses on some specific clinical conditions (renal disease, diabetes and asthma) as well as general immunocompetence and the potential to attenuate associated oxidative stress via dietary intervention. Finally the implications of current knowledge on the recommendations for vitamin E and taurine in therapeutic diets are evaluated.

Introduction to oxidative stress and relation to disease

Free radicals or reactive oxygen species (ROS) are produced continuously in mammalian systems as a consequence of normal metabolic processes. Other exogenous sources of ROS include exercise, pollution, sunlight and drugs (including anaesthetics). Although these compounds have an important role in normal physiological mechanisms, when ROS production is excessive the result is oxidative stress. This is the term usually applied to the outcome of oxidative damage to biologically important molecules such as protein, lipid and nucleic acids. Proteins have long been known to be susceptible to oxidation by free radicals or reactive oxygen species (ROS), and aromatic amino acids, cysteine and disulphide bonds are particularly vulnerable. All biological materials contain a variety of polyunsaturated fatty acids, which are predominantly located in membrane lipids. They are highly susceptible to ROS damage – particularly by the superoxide anion, which abstracts hydrogen atoms from the methylene interrupted double bond structure.

The group of compounds known as antioxidants (sometimes referred to as 'free radical scavengers') are the major defence against oxidative stress, and function to protect membrane and cytosolic components against free radical damage. There are many antioxidants present in mammalian systems including primary antioxidants, which prevent the formation of new radical species. These comprise enzyme systems such as superoxide



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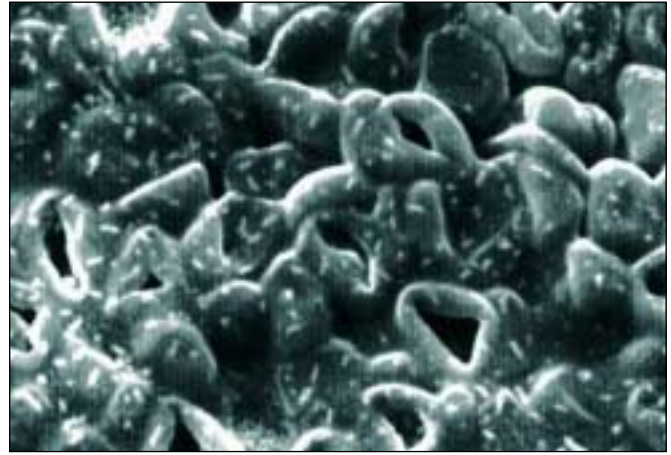
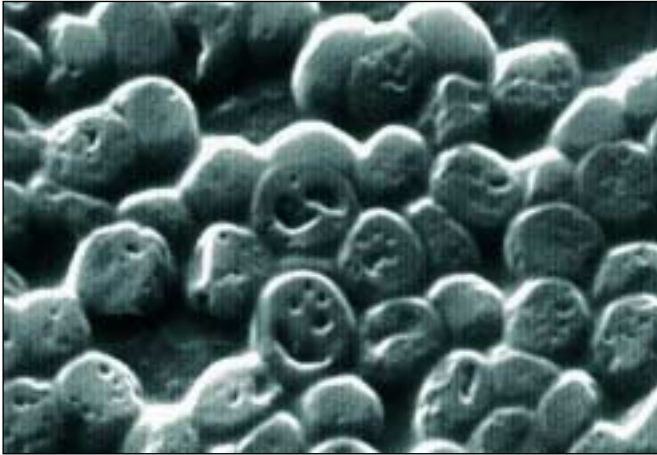


Figure 1 Canine erythrocytes – left, normal erythrocytes; right, erythrocytes oxidatively stressed in vitro.

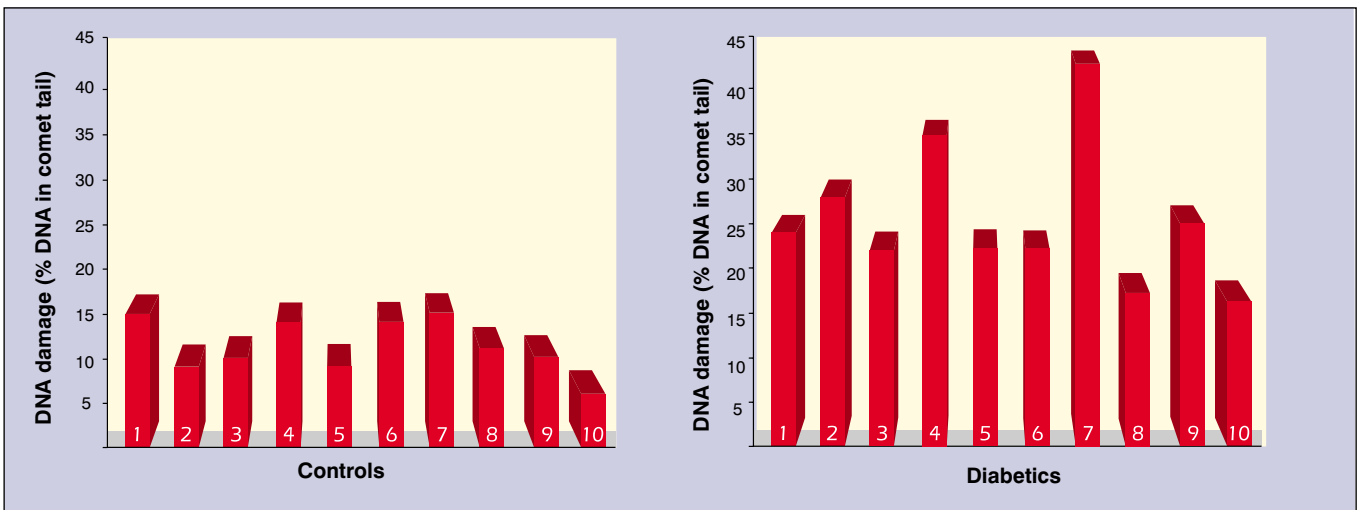


Figure 2 DNA strand breaks in lymphocyte from healthy controls and diabetic humans.

dismutase (SOD) and glutathione peroxidase (GSH Px). Secondary antioxidants trap radical species thus preventing chain reactions, and include nutrients such as vitamin E, vitamin C and taurine. Carotenoids such as β -carotene also trap radical species. The final line of antioxidant defence is provided by the repair systems such as the enzyme methionine sulphoxide reductase that regenerates methionine residues within oxidised proteins and restores function.

The role of vitamin E

Vitamin E is a collective name for eight naturally occurring tocopherols and tocotrienols, of which α -tocopherol is biologically and chemically the most active. It is a potent antioxidant, which resides primarily in biological membranes, serving to protect membrane phospholipids from peroxidation. Historically, as with all nutrients, there has been little interest in dietary vitamin E levels above those required to prevent signs of deficiency in cats and dogs. The minimum requirement has been established as 1.4 IU α -tocopherol/MJ diet for adult cats and dogs (1, 2). However not only does the amount required to prevent deficiency vary depending on other dietary ingredients, e. g. polyunsaturated fatty acids, vitamin A and selenium, but the physiological condition of the animal also contributes to the vitamin E requirement. Generally metabolic stress resulting from trauma or infection increases the vitamin E requirement.

The role of taurine

Taurine, a sulfonated β amino acid obtained through diet or derived from methionine and cysteine metabolism, is generally considered to be a non-essential dietary component for most species. The exceptions of course are carnivorous mammals, including the cat, where the essentiality of dietary taurine has been recognised since the 1970s (3). Human nutritionists have traditionally discounted taurine as a nutrient, primarily because humans have considerable taurine body pools. As the role of taurine in membrane stabilisation, antioxidant defence, maintenance of calcium homeostasis and growth modulation have become clearer, however, taurine has been reclassified as a conditionally essential nutrient. Studies have demonstrated that a nutritional requirement exists for taurine in human infants and in adults receiving long term total parenteral nutrition (4). Decreased plasma taurine has been identified postoperatively and in pathologic states including trauma and sepsis (5). Depending on the system of interest, taurine has been demonstrated to act as both a primary antioxidant that scavenges free radicals and a secondary antioxidant that attenuates oxidant-induced changes in membrane stability (6).

The potential role of antioxidants in disease

Diabetes mellitus

Diabetes in humans is a disease associated with increased oxidative stress.



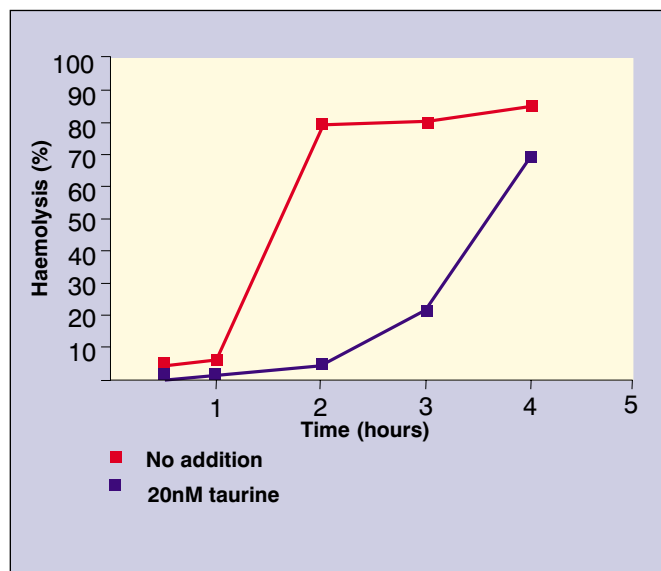


Figure 3 Effect of taurine on the suppression of induced damage using canine red blood cells.

The cause of this is not yet fully understood but is thought to include mitochondrial dysfunction, direct enzyme inhibition by hyperglycaemia, auto-oxidation of glucose and activation of NADPH-oxidase. The oxidative stress manifests itself as elevated concentrations of lipid peroxidation products, erythrocyte fragility (Figure 1) and decreases in the antioxidant enzyme systems (CAT, GSH-PX, SOD) (7, 8). Recent studies have also shown a positive correlation between blood glucose concentration and oxidant-induced lymphocyte DNA damage (9) (Figure 2). Clinical intervention to control blood glucose concentrations may alleviate oxidative DNA damage, although this has not been reported. Likewise, dietary intervention with antioxidant nutrients, vitamins E, C and taurine may mediate the high level of oxidative stress in diabetics. Certainly, steady state estimates of cellular DNA oxidation indicate a role for antioxidant vitamins in the prevention of DNA oxidative damage. In one study, a combined dietary supplement of 25 mg β -carotene, 100 mg vitamin C and 280 mg vitamin E resulted in a significant decrease in lymphocyte DNA strand breaks over a 4 month period in healthy adult humans (10). Similar doses of vitamin E in cats and dogs equate to approximately 10 times the current minimum requirement (1.4 IU/MJ diet). Other antioxidant intervention studies have reported decreased activity of phospholipase A2 and associated lipid peroxide formation, as well as attenuation of indices of oxidative damage in the diabetic precataractous lens via taurine supplementation. This is particularly interesting in cats, where there are anecdotal reports of central retinal degeneration in diabetic cats with apparently normal plasma taurine levels. It is possible, through dietary intervention, to increase the lens taurine concentrations in diabetic rats although the dietary concentrations used have been in the order of 5% (31). This is equivalent to 5 g/100 g compared to typical dietary levels of 100 to 500 mg/100 g.

Renal disease

Chronic renal disease is a condition that is accompanied by an acceleration of oxidative events. ROS have been implicated in the genesis of different forms of renal disease, predominantly experimentally-induced glomerulonephritis but also in different forms of acute renal failure. Studies have revealed that human subjects with chronic renal failure (CRF) frequently have unusually low plasma levels of vitamins E and C (11). This is in spite of adequate dietary intake, suggesting increased utilisation of these particular antioxidants. Likewise, markers of lipid peroxidation are

significantly higher in the plasma and erythrocytes of patients with CRF (11). The mechanisms by which CRF induces oxidative insult are undefined, but it is clear that renal mass reduction contributes to oxidant-induced glomerular and tubular injury. Intervention studies in rats using a remnant kidney model have indicated that the prevention or inhibition of such injuries may occur with dietary vitamin E supplementation. It appears that vitamin E can modulate tubulointerstitial injury and glomerulosclerosis, lower the elevated expression of transforming growth factor- β 1 and reduce markers of lipid peroxidation in plasma and renal tissue (12). This suggests a potential utility of vitamin E in slowing progression of glomerulosclerosis and tubulointerstitial injury. Other rat studies have identified a potential role for vitamin E intervention in attenuating age-related kidney dysfunction. In a group of elderly rats maintained for 9 months on a diet containing 50 IU/kg, ageing was accompanied by a 60% reduction in GFR, a 3-fold increase in F2 isoprostanes (markers of lipid peroxidation) and an increase in advanced glycosylation end products (AGEs). A treatment group maintained on 5000 IU/kg diet showed a 50% increase in GFR, suppression of F2 isoprostanes and AGE, as well as a tendency for attenuation of glomerular sclerosis (13). The dosage in the supplemented group is approximately equivalent to 200 times the minimum dietary requirement for cats and dogs (1, 2). Whether a more moderate dose would be equally efficacious is unclear.

Asthma

Feline asthma closely parallels human asthma, another clinical condition now known to be associated with oxidative stress. Although the pathogenesis of asthma, both human and feline, is not fully defined, a typical feature is an increase in the number of inflammatory cells in the lung. Such cells generate ROS, which are involved in the pathophysiology of asthma, including airway smooth muscle contraction, increased airway reactivity and increased vascular permeability (14). Studies have indicated that there is reduced activity of SOD in the lung cells of asthmatics. SOD activity is reduced by 25% in bronchoalveolar lavage cells and by almost 50% in bronchial epithelial cells (14). It has also been demonstrated that cells both of peripheral blood and lung from asthmatics generate increased ROS and this increase correlates with disease severity. Despite the evidence implicating oxidative insult in the development of asthma, there are virtually no reported antioxidant intervention studies. *In vitro* studies have demonstrated that taurine can protect against bronchiolar damage induced by NO₂ (15). Complementary *in vivo* rodent studies have confirmed that taurine at physiological concentrations (1%) protects mammalian alveolar pneumocytes following exposure to acute free radical insult, preventing both the initial acute inflammatory response and the later development of fibrosis (16).

Effect of antioxidants status on immunological function

The immune system is particularly sensitive to oxidative stress, primarily because immune cells rely heavily on cell to cell communication to work effectively. Peroxidation of cell membranes compromises membrane integrity and disrupts intracellular signalling. Since vitamin E is the most potent chain breaking antioxidant present in cell membranes, its potential to maintain membrane integrity and hence promote immune cell function has been well studied. In human and rodent studies, supplementation to levels of 10–50 times the minimum requirement of vitamin E is reported to enhance immunological function. Studies in humans have shown that supplementation of 800 IU/day improves the immune response, as measured by delayed-type hypersensitivity tests and lymphocyte proliferation in response to concanavalin A (17). Rodent studies indicate

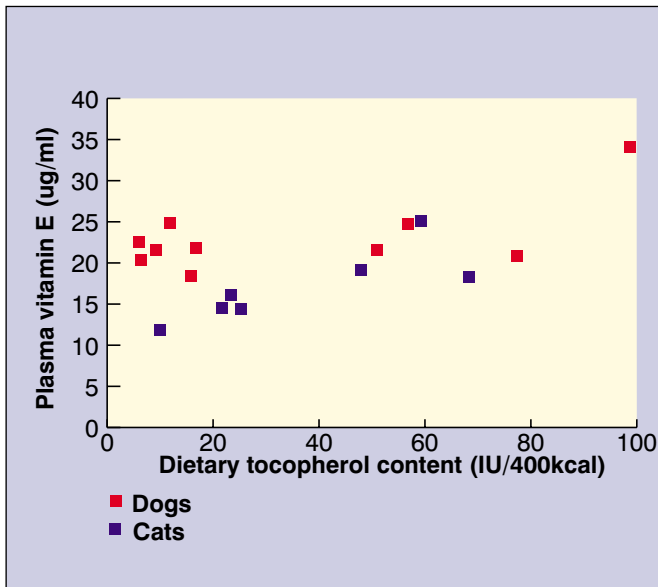


Figure 4 Canine and feline plasma response to dietary vitamin E content. The above data are derived from a series of studies with healthy adult cats and dogs. No significant effect of age or gender on plasma tocopherol was observed. The data indicate a clear plasma dose response to dietary vitamin E in cats. Typical baseline values are in the range of 10 to 15 ug/ml, and can be increased by dietary supplementation. The data are less clear for dogs, where there is indication of a dose response but the baseline values tend to be higher. The reasons for this are unclear but may reflect a breed effect.

increased antibody production (particularly IgA, IgG and IgM) and specific antibody production as well as increases in the mean cell size of B1 and B2 lymphocytes, when dietary vitamin E levels are enhanced (18, 19). The mechanisms for the varying immunopotentiating effects of vitamin E have yet to be clarified but it is likely that the effect is through the ability of vitamin E to maintain the functional integrity of immune cells, in particular neutrophils.

Numerous roles of taurine in immune cell function have been identified. Taurine is present in high concentrations in proinflammatory immune cells including lymphocytes (where it constitutes more than 50% of the free amino acid pool) and neutrophils (20). Taurine is known to modulate neutrophil activity through interaction with the respiratory burst enzyme myeloperoxidase (MPO) and also attenuates the age-related decline in T lymphocyte proliferation (21, 22). Like vitamin E, it is postulated that taurine helps maintain immune cell function via its ability to maintain cellular integrity. *In vitro* studies with canine erythrocytes have indicated that taurine confers increased resistance to haemolysis (23) (Figure 3). However, few studies on taurine and immune function have been reported.

Recommended dosages

Studies in humans have indicated that plasma concentrations of tocopherol can maximally be raised by two- to threefold irrespective of the magnitude of dosage (24). Equivalent studies in cats and dogs also indicate that, typically, plasma saturation is in the order of double or triple baseline values (25). Nonetheless, evidence from human studies indicates that a measurable benefit of dietary intervention with vitamin E can be achieved when baselines are only moderately increased. Evidence of reduced lipid peroxidation has been demonstrated when the response to vitamin E has been defined as plasma increases in the order of 1.3–2.0 (26). On this basis, to achieve a 50% increase in the mean plasma vitamin E

concentrations reported for cats and dogs (14.9 ug/ml and 20.28 ug/ml respectively), a dietary level of between 25 and 50 IU/400 kcal is recommended (Figure 4). There is currently little rationale for supplementation beyond this and, taking into account safety data, a dietary increase in the range of 10 to 20 times the minimum nutritional requirement is considered prudent.

In cats, to maintain plasma taurine levels in the normal range (> 60 mmol/l), a dietary requirement of 400 mg/kg has been defined (2). In practical terms this means that a canned diet must supply at least 39 mg of taurine/kg bwt per day, and a dry diet at least 19 mg/kg bwt per day (27). With a built in bioavailability factor, this equates to around 250 mg/400 kcal in a canned food and 100mg/400 kcal in a dry food. There is a well-documented linear plasma response to dietary taurine and to achieve a twofold increase in circulating levels it is recommended that these target levels be doubled. There is little information on taurine pharmacokinetics in dogs but a similar target is suggested.

Safe levels of supplementation

The maximum tolerable level of vitamin E for cats and dogs has not been defined, although human data indicate a level of up to 1000 IU/day is safe (28). A presumed safe level of 75 IU/kg bwt/day has been suggested for all animal species (29). For cats, assuming an average 4 kg bwt this is equivalent to a daily intake of 300 IU (around 500 IU/400 kcal). For a dog weighing 30 kg it equates to a daily intake of 2250 IU. The risk of excessive vitamin E consumption is relatively poorly defined, but in the absence of supplementary vitamin K, the potential to interfere with blood clotting mechanisms has been reported. However, cats maintained for 32 weeks on diets that delivered plasma vitamin E concentrations of 16.0 and 11.1 ug/ml respectively showed no significant difference in terms of blood clotting time (30). Equivalent information is lacking for dogs, however recent data indicate that a daily dose of 1000 IU in adult greyhounds may have adverse effects (31). This manifested as significantly reduced race times and may be a consequence of compromised vitamin K status, or a pro-oxidant effect of excessive tocopherol. As recommended above, a safe and effective supplement should be in the range of 10 to 20 times the minimum requirement. This should ensure that vitamin E status is optimised, without any risk of adverse effects. Taurine, along with the majority of amino acids, is considered to be safe at very high dietary intakes. No upper safety limit exists for the inclusion of taurine in cat and dog foods and therefore dietary inclusion levels of up to 500 mg/400 kcal present no concerns.

Conclusions

There are many dietary antioxidants which, when supplemented in cat and dog foods, may confer a benefit. For the purposes of this review, however, the focus has been on two specific antioxidants whose efficacy is supported by a wealth of literature. The relative safety of both these nutrients, even at extraordinarily high dietary inclusion levels, adds to their attraction as adjuncts to conventional therapeutic diets. Given the range of clinical conditions that are associated with increased oxidative stress, the benefits of antioxidant therapy are probably relevant to the majority of cats and dogs



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