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Antioxidants in Veterinary Nutrition

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Nutritional molecules with antioxidant properties have long been recognized, with vitamin E perhaps being the prototypical molecule in this class. The discovery of a fat-soluble factor, vitamin E, that supported reproduction in rats was first described in 1922 by Evans and Bishop [1]. Shortly thereafter, Olcott and Mattil [2] reported that this factor also possessed antioxidant properties. Evans coined the term *tocopherol* to describe the factor that comes from the Greek *tokos* (childbirth) and *pherein* (to bear).

Much like its name origin, the potential health benefits of tocopherol seem to have multiplied and may perhaps be unrivaled by any other nutritional molecule. Many of these claims have been birthed by scientific hypothesis, anecdotal reports, or, in some cases, sound scientific study. Whatever the origin of the purported benefit, intense debate often follows about the true scientific validity of the benefit.

Despite the intense debate surrounding benefits of vitamin E and other antioxidant-like molecules, significant scientific progress has been made in trying to define the complexities of nutritional antioxidants. It is the purpose of this article to try and define what a nutritional antioxidant is, how it functions in the body, and how to measure those benefits. Necessary to this discussion is a review of the free radical theory of aging because it directly interfaces with the proposed benefits of nutritional antioxidants. Finally, the scientific literature with specific application to dog and cat nutrition is reviewed.

DEFINITION OF ANTIOXIDANTS AND FREE RADICALS

Chemically speaking, antioxidants and free radicals derive their terminology from the field of electrochemistry. The loss of electrons from a substance is called oxidation, and the gain of electrons is referred to as reduction. An alternative terminology is to call a substance that donates electrons (it is being oxidized) to another substance a reducing agent and the acceptor of electrons (it is being reduced) an oxidizing agent. The oxidizing agent is always being reduced in a reaction, and the reducing agent is always being oxidized. When oxidation and reduction take place in the same chemical equation between two

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substances, it is termed a *redox reaction*. The spontaneity of redox reactions is defined by physicochemical properties determined by thermodynamics, which can be quantitated by the Nernst equation. In general, the balance of this potential energy equation is a measure of how willing a molecule is to give up an electron as compared with its willingness to accept an electron in relation to the hydrogen half-cell.

One proposed definition of an antioxidant follows: an antioxidant is any substance, which when present in low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate [3]. In serving this function, the antioxidant may preserve the structural integrity or function of a biologic molecule, and thus preserve its function in the cell. Nevertheless, this concept may be too simplistic, because it has been shown that some cellular signaling pathways seem to depend on redox chemistry to manifest their "normal" biologic function.

Free radicals are chemically unstable molecules that have an unpaired free electron. Most commonly, oxygen free radicals are used as examples, but other molecular species may also exist as free radicals. Oxygen free radicals are used as the prototypical molecule for the remainder of this discussion because they are perhaps the most biologically relevant.

The existence of an unpaired electron creates a thermodynamically unstable situation. As such, the molecule desires to gain (reduction) or to lose (oxidation) an electron to achieve a more thermodynamically stable state. Thus, a free radical may act as an oxidizing agent or a reducing agent depending on its thermodynamic propensity for stability. For example, superoxide is a normal byproduct of cellular respiration and is represented by the formula $O_2^{-\cdot}$. Superoxide is favored by thermodynamics to lose an electron to become oxygen, and eventually water, via a hydrogen peroxide intermediate. Conversely, hydroxyl radical, $^{\cdot}OH$, has a strong preference to gain an electron (oxidize other molecules) to achieve an OH^- configuration. Thus, the chemistry of free radical reactions, with the reactant passing or gaining electrons, is complex. This chemistry depends not only on which species of free radical is generated in vivo but on where the generation of that molecule is located in the subcellular portion of the cell. For example, a highly reactive species produced in the mitochondria is unlikely to diffuse into the cytoplasm. Alternatively, a less reactive species, such as hydrogen peroxide, may diffuse into the cytoplasm before being chemically engaged in a redox reaction.

Redox and free radical chemistry reactions may occur via direct uncatalyzed means or may be catalyzed by other molecules and/or metals or proteins acting as enzymes. In addition, these systems may work in networks that are dependent on the proximity and species of redox coupling required. For example, superoxide is produced as a normal byproduct of cellular respiration in the mitochondria. Under normal cellular conditions, electrons "leak" from the electron transport chain, converting approximately 1% to 3% of oxygen molecules into superoxide. Cells have a multitude of mechanisms to detoxify free radicals, and in this case, they use a two-step enzymatic method. In the first

step, superoxide free radical is simultaneously reduced and oxidized (dismutated) by superoxide dismutase to form hydrogen peroxide and oxygen (reaction 1). Although hydrogen peroxide is also a reactive oxygen species (ROS), it is much less reactive than superoxide to the point where it may diffuse out of the mitochondria before reacting with another molecule. In the second step, hydrogen peroxide is converted into water and oxygen by catalase enzymes (reaction 2). Ironically, the most mutagenic of the ROS, hydroxyl ($^{\cdot}OH$) free radical, is generated as a consequence of disabling superoxide to hydrogen peroxide. Termed *Fenton chemistry*, peroxide readily reacts with ferrous iron (Fe^{2+}) or other transition metal ions to produce hydroxyl radical (reaction 3). By the same token, ferric iron (Fe^{3+}) can accept an electron from superoxide, cycling it back to the ferrous state and making it available to react with another peroxide molecule (reaction 4). Thus, even trace amounts of iron ion can potentially catalyze the formation of large amounts of hydroxyl free radical:

$$2O_2^{-\cdot} + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2 \quad (1)$$

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \quad (2)$$

$$H_2O_2 + Fe^{2+} \longrightarrow {}^{\cdot}OH + OH^- + Fe^{3+} \quad (3)$$

$${}^{\cdot}O_2^- + Fe^{3+} \longrightarrow O_2 + Fe^{2+} \quad (4)$$

When these individual reactions are linked together in a biologic system, a more dynamic metabolic picture of potential pathways can be viewed (Fig. 1). From this more integrated picture, it can be seen that the production of free radicals is dependent on multiple pathways and the availability of detoxification mechanisms versus reactive materials. From this dynamic balance, some have referred to the relative overproduction of oxidative and/or reactive materials produced, as compared with the detoxification pathways, as oxidative stress.

The hydroxyl radical is highly reactive, oxidizing most organic compounds at almost diffusion controlled rates ($K > 10$ mol/L/s) [4]. Because of its high reactivity, it is indiscriminate, reacting with the first substrate available. It therefore has high destructive and mutagenic potential. Membranes of the mitochondria are particularly susceptible, because the radicals are formed in close proximity.

From this example, it is evident that redox reactions are quite complicated and may rely on a multiplicity of reactions to achieve an overall end result. It has been recently proposed that antioxidants may work in even more complex networks than the prior example, where several different steps or cellular components are required to achieve successful detoxification of an oxidizing agent. An example of this is given in Fig. 2, where an oxidant is produced and then detoxified by a series of stepwise reactions.

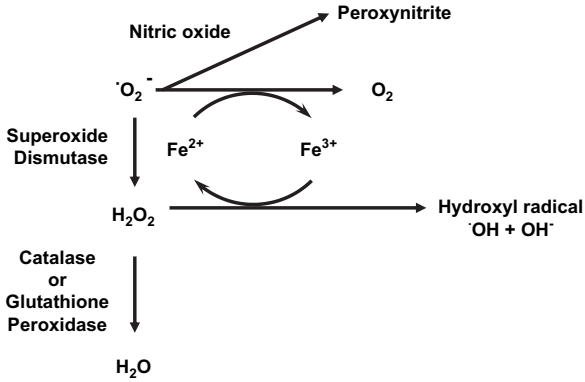

Fig. 1. Metabolism schemes of superoxide anion.

FREE RADICAL THEORY OF AGING

The free radical theory of aging was first proposed in 1956 by Harman [5]. The theory postulates that ROS produce cellular damage and that age-dependent pathologic changes may be a resultant cumulative response to these alterations. It is now generally accepted that the main source of ROS in mammals is from aerobic respiration byproducts in the mitochondria [6]. Accordingly, one interpretation of the free radical hypothesis of aging would predict that aging should be slowed, and possibly even reversed, by decreasing the effects of ROS.

This hypothesis has led to many strategies to try and mitigate the effects of ROS. One highly touted strategy is to increase the capacity to suppress ROS effects via antioxidants or antioxidant defense mechanisms through nutritional supplementation. The effectiveness of this seemingly simple strategy is dependent on a wide range of different biologic factors.

DETERMINANTS OF AN EFFECTIVE NUTRITIONAL ANTIOXIDANT

In consideration of the previous stated theory, it would seem straightforward to assume that the addition of antioxidants to a biologic system should result in positive effects in reducing the aging process. Many intervention studies have met with limited or contradictory results compared with this intended outcome, however. Many possibilities exist for why this may be true, and a few are discussed here.

Distribution and bioavailability of antioxidants are important determinants of biologic outcome and cannot be overlooked. It is important when evaluating potential antioxidant interventions to understand potential limitations to application. For example, several plant flavonoids and other polyphenols have limited solubility and absorption in the gut as compared with other water- or fat-soluble compounds [7]. Even antioxidants considered to be easily absorbed and distributed may have marked bioavailability depending on other

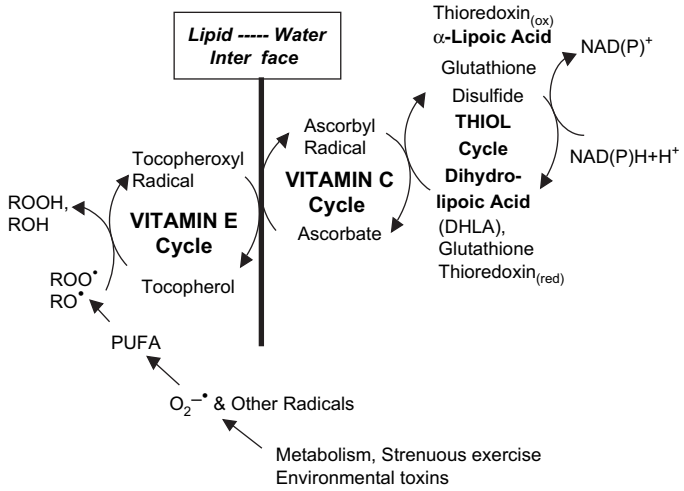

Fig. 2. Example of an antioxidant network to detoxify free radicals.

physiologic factors, such as food intake and composition [8,9]. This report showed that absorption of vitamin E was least effective from gel capsules given without a meal and variably effective when given with a meal. Application of vitamin E adsorbed onto a cereal provided consistently higher rates of bioavailability, however. This type of study highlights the importance of monitoring and extraneous factors that may affect bioavailability.

Metabolic transformation may alter the biologic activity or distribution of orally administered antioxidants differentially between species. For example, cats lack the enzyme, β-carotene 15,15′-dioxygenase, that cleaves β-carotene (provitamin A) into two molecules of retinal, whereas the activity of this enzyme is relatively high in herbivores [10]. Thus, carotenoids are more likely to be absorbed intact in cats, and possibly other carnivores, whereas they serve relatively more of a previtamin A function for herbivores. Another example is α-lipoic acid, which has been shown to have different rates of metabolic elimination in cats and other species. Cats metabolize and eliminate lipoic acid at a much slower rate than other species [11]. Finally, another functional consideration in this discussion is the effect of age itself. Although vitamin C is not considered essential for rats, it has been shown that as rats age, the metabolic enzymes responsible for recycling and transport of vitamin C in hepatocytes become impaired, which, if severe, may impart a state of conditional essential status for vitamin C to the aged rat [12,13].

NONCLASSIC MECHANISMS OF ACTION OF CLASSIC ANTIOXIDANTS

This discussion has focused on classic definitions and mechanisms of actions of antioxidants. Research in the past 10 years has revealed that many of these

"antioxidant" molecules have other important physiologic functions. Some functions include but are not limited to antioxidants as regulators of second messengers, cell cycle signaling, and control of gene expression through a variety of mechanisms. These findings may be considered extensions of the classic antioxidant action, but it is clear from several lines of evidence that these aspects of redox status in the cell are well regulated and coordinated in such a manner that they probably are inherent in the design of cellular function and outcome rather than random in nature.

Resveratrol, a polyphenol from red grapes, has been shown to activate sirtuin 2, a member of the sirtuin family of nicotinamide adenine dinucleotide (NAD)+-dependent deacetylases, which mimics the effects of caloric restriction and results in prolongation of cell life [14]. Insulin signaling has been shown to be mimicked by hydrogen production, and it now recognized that this is a component of insulin signaling physiology [15]. Nuclear factor (NF)-κβ signaling of apoptosis has been shown to be activated by an alternative pathway via hydrogen peroxide, and specific mitochondrial-targeted antioxidants have been shown to alter this signaling pathway [16–18]. NF-κβ is not the only transcription factor considered to be redox sensitive, because several other factors have been characterized with these properties in the past several years [16,19]. As one can see from these few examples, the roles and physiology of antioxidant molecules are far reaching and go beyond classic chemistry understanding of previous years. It is also easy to see from these recent reports why effects of interventions have sometimes been contrary and difficult to interpret.

HOW TO MEASURE OUTCOMES OF STUDIES WITH ANTIOXIDANTS

Controversy and difficulty have developed in interpreting the vast number of studies with antioxidant supplements. As evidenced previously, the biologic effects of antioxidants may take place by way of multiple divergent or convergent pathways, thus making interpretation difficult. Also, because the effects of free radicals are supposed to be insidious and temporally delayed in taking effect, prediction of long-term outcomes from short-term experiments has proven to be a challenge. Finally, determining the outcome event of importance is also problematic, based on the variety of end point measures that have been developed to measure the effects of antioxidants. The advantages and disadvantages of some measurement outcomes are briefly discussed here so as to highlight some potential pitfalls of current methodology.

Increased Concentrations of Antioxidants in Foods and Tissues

Oral antioxidant administration as a supplement or in combination with a food does not ensure absorption and distribution into tissues. Some antioxidants are more readily absorbed than others and may display species differences in absorption, as discussed previously. Vitamin E is usually much easier to absorb than some water-insoluble plant phenols; yet, even vitamin E displays variable absorption and distribution depending on many factors. As discussed

previously, the absorption of vitamin E was observed to be more efficient when administered with a meal [9]. In addition, vitamin E depletion and repletion seem to have different kinetic parameters depending on tissue type [20,21]. This highlights that the fact that absorption and distribution of oral antioxidants must be relevant to the target tissue and intended biologic outcome. Nonetheless, the variability in bioavailability and distribution has not limited the number of studies linking increased ingestion or increased serum values of antioxidants to a variety of health outcomes in various target tissues. If mere absorption and distribution do not prove causality, then what measurements are available to start developing mechanistic arguments for biologic efficacy?

Decreased Markers of Free Radical Damage

Free radicals are short lived and difficult to measure as to their native species. In lieu of measuring free radicals directly in target tissues, a variety of new laboratory methods have been developed that measure biologically stable molecules produced by free radical chemical reactions as markers of free radical production in a biologic system. If these markers increase in serum or tissue, it is presumed that there are more free radicals being produced, and thus more damage. If they decrease, it is presumed that the production of free radicals has been decreased. These markers are specific for different biomolecules, such as DNA (8-oxodeoxyguanosine), lipids (eg, alkenyls, malondialdehyde [MDA], thiobarbiturate reacting substances [TBARS]), prostaglandins (isoprostanes), protein (eg, nitrotyrosine, protein carbonyls), and advanced glycation end products (AGEs). The utility of these measurements has been debated because they are indirect measures of presumed free radical reactions, sometimes in distant tissues. As such, they are responses to oxidative events but do not provide direct mechanistic effects of antioxidant action in target tissues.

The next modality of investigation is to look directly at target tissue effects of orally administered antioxidants. Certainly, these studies can provide biochemical information on mechanisms in the tissue of interest as compared with the indirect measures discussed previously. This type of research has yielded interesting results with a variety of antioxidants. For example, aged rats, a vitamin C–independent mammal, have a decreased ability to recycle vitamin C in their hepatocytes, which may be restored by the administration of lipoic acid and acetyl-carnitine [12]. Aged rats had increased oxidative damage to their hepatic proteins, which imparted decreased enzymatic activity and more susceptibility to protein degradation [22]. Finally, it has been shown that oxidative damage increased in brains of aging Beagles and rats and that the damage was correlated with memory loss in the rats. [23,24]. Intervention with acetyl-carnitine and lipoic acid partially reversed the memory loss in the older rats [24].

Intervention Trial Outcomes of Antioxidant Interventions

These studies are much more difficult to perform, because the expense, length of time required for intervention, and ability to control the dietary intake of individuals is problematic. Use of animals with shorter life spans than human beings is useful in developing strategies that may be beneficial in characterizing

these outcomes, however. It is presumed that the shorter life span models may show accelerated aging attributable to more rapid free radical damage; thus, interventions may be assessed more quickly [25]. In addition, specific genetic models, such as the senescent accelerated mouse that overproduces free radicals, and transgenic models are becoming more available and may lend some insight into the efficacy and modes of action of different dietary antioxidant supplement regimens.

APPLICATIONS TO VETERINARY NUTRITION

As one can see, the science of nutritional antioxidants has advanced over the past several years. Numerous studies have revealed new and important biologic benefits of supplementing foods or diets with oral antioxidants in a variety of species. The next question of interest is what is the body of evidence available to assess in the veterinary literature and what does it mean for the practitioner? In an effort to answer this, we examine the literature on mainstream antioxidants as they apply to canine and feline nutrition.

Vitamin E

Canine

Requirements for vitamin E in dogs and cats were suggested as early as 1939 and were modified based on selenium (Se) and polyunsaturated fatty acid (PUFA) content of foods in the 1960s [26–29]. From the published research, the National Research Council (NRC) states that the requirement for dogs should be met by vitamin E at a rate of 22 IU/kg of diet (based on a diet of Se at a rate of 0.1 ppm, not more than 1% linoleic acid, and metabolizable energy [ME] at a rate of 3670 kcal/kg). This results in a range roughly equivalent to 0.4 to 1.4 IU/kg of body weight for maintenance of pregnant and/or lactating dogs [30].

Effects of vitamin E on other biologic outcomes have been tested in dogs, and it has been found that levels higher than the requirement may confer targeted biologic benefits. Increasing the dietary intake of vitamin E up to 2010 mg/kg of diet (dry matter basis) in geriatric Beagles has been shown to improve immune function [31,32]. Increased intake of vitamin E in food is directly related to increased vitamin E content of skin, which may provide health benefits for targeted disease processes [33]. Vitamin E concentrations in blood decrease with exercise, and higher levels have been associated with improved performance [34,35]. Finally, vitamin E has been shown to provide protection from damage by ischemia in a variety of tissues [36–38]. There are no published toxicity data for vitamin E in dogs; however, concentrations exceeding 2000 IU/kg of dry matter of food have been fed for 17 weeks without observable negative reactions [31]. An upper limit of toxicity has not been documented; yet, a level of 1000 IU/kg of food (dry matter basis), or 45 IU/kg of body weight, has been suggested [27].

Feline

Foods for cats are often higher in fat and polyunsaturated fats, which may provide a different matrix reference for determining requirements. Nonetheless,

several studies have shown that the amount of vitamin E needed to support growth and reproduction in cats is in the same general range as in dogs when adequate Se and excessive PUFAs are accounted for. Thus, vitamin E at a range of 0.5 to 1.7 mg/kg of body weight has been suggested by the NRC [39] for maintenance and pregnancy and/or lactation, respectively.

Vitamin E supplemented to food at 272 and 552 IU/kg of food (dry matter basis) resulted in improved immune function in aged cats [29]. Supplementation of D-α-tocopherol to cats at a dose of 1000 IU enhanced neurologic recovery in a model of spinal cord compression [26]. Supplementation of vitamin E at a dose of 800 IU/d via gel caps showed no difference from control in prevention of onion powder– or propylene glycol–induced Heinz body anemia [40]. Vitamin E and cysteine supplemented to food (vitamin E at a rate of 2200 U plus cysteine at a rate of 9.5 g/kg of food) showed protective effects on acetaminophen-induced oxidative production of methemoglobinemia [41]. Also, pretreatment of cats with a combination of vitamin E and Se (vitamin E at a rate of 200 IU plus Se at a rate of 50 μg) for 5 days delayed motor nerve degeneration in a model of axonal degeneration [42]. A presumed safe upper limit (SUF) for oral administration has not been established; however, administration of vitamin E parenterally at 100 mg/kg of body weight to kittens resulted in significant mortality [43].

Vitamin C

Dogs and cats

Dogs and cats are considered capable of synthesizing required amounts of vitamin C needed by de novo mechanisms [44,45]. Chatterjee and colleagues [46] showed that hepatic in vitro synthesis of vitamin C in dogs and cats was much less (10%–25%) than that in other mammals, leading to speculation that the ability to synthesize vitamin C may be limited; however, no follow-up work has been performed. The pharmacokinetics of vitamin C administration in dogs showed that ascorbic acid and ester-C were rapidly absorbed and may possibly use an active transport mechanism in the gastrointestinal tract [47].

The subchronic intravenous toxicity of a median lethal dose (LD_{50}) has been reported to be greater than 500 mg/kg/d in cats and greater than 2000 mg/kg/d in dogs [48]. Supplementation of vitamin C (0, 200, 400, or 1000 mg/d) to cats resulted in a small progressive reduction in urine pH [49]. It has been noted in human beings that intake of ascorbate at the upper recommended limit of 2000 mg/d increased urine oxalate excretion and stone risk [50]. Moderate supplementation of vitamin C in healthy cats up to 193 mg/kg of food (dry matter basis), approximately 2 mg/kg of body weight, did not seem to increase the risk of oxalate stone formation in urine. However, because vitamin C may be converted to oxalate, higher levels may increase oxalate excretion in the urine [51]. Supplementation of vitamin C to rats at 1500 mg/kg of food (dry matter basis) may decrease erythrocyte fragility when vitamin E is near the requirement level in the diet [52]. Additionally,

it has been reported that oral supplementation at 1 g/d may slow race times in Greyhounds [53].

β-Carotene and Other Carotenoids

Some work has been performed on the use of carotenoids, predominantly β-carotene, in canine and feline nutrition. As mentioned previously, β-carotene can serve as a precursor to vitamin A in the dog but not in the cat. Interestingly, although carotenoids possess antioxidant properties, most of the research in dogs and cats has focused on immunomodulatory benefits.

Supplementation with β-carotene has been shown to increase concentrations of β-carotene in the plasma and white blood cells of cats and dogs [54,55]. The concentrations reached in the plasma of cats are approximately 50-fold higher compared with those of dogs at the same approximate time and dose rate, however, indicating that most of the β-carotene administered to dogs is probably converted to vitamin A rather than absorbed directly as β-carotene (Table 1). Human beings convert approximately 60% to 75% of β-carotene into vitamin A and absorb approximately 15% intact. With this information, it is interesting to note that the mean concentration of β-carotene in serum from human beings is approximately 0.3 μmol/L, which is approximately 10-fold greater than concentrations observed in supplemented dogs. Nonetheless, supplementation of dogs with β-carotene has been reported to improve immune function in young and aged animals [56,57].

Supplementation of the carotenoid lutein has been shown to increase plasma and leukocyte concentrations of that ingredient in dogs and cats. In addition, improvement in the immune function of both species has been reported with lutein supplementation of the food [58,59]. A novel form of astaxanthin has been shown to provide cardioprotection from vascular occlusion in dogs [60].

Carotenoid safety, as β-carotene, has been evaluated in Beagles at extremely high doses from 50 to 250 mg/kg/d administered orally in the form of beadlets [61]. Although discoloration of the hair coat and liver vacuolization were noted at all levels, no consistent findings of toxicity were found. Carotenoid safety is not well evaluated in cats but could be presumed to be safe based on wide margins of safety in other mammals and lack of conversion to vitamin A. Nonetheless, canthaxanthin supplementation to cats for 6 months induced retinal pigment epithelial changes and some vacuolization but no functional electroretinogram changes [62].

Table 1
Concentration in blood of cats and dogs supplemented with β-carotene for at least 7 days

Species	Dose	Body weight	Peak plasma concentration
Cat	10 mg/d	3–3.5 kg	0.95 μmol/L at 7 days
Dog	25 mg/d	7–9 kg	~0.02 μmol/L at 7 days

Selenium

Se was first recognized as an essential nutrient in 1957 based on its ability to spare vitamin E in exudative diathesis in chicks [63]. The metabolic basis for its nutritional function remained unclear until it was discovered in 1973 [64] that Se was a component of glutathione peroxidase (GSHpx). Subsequent discoveries revealed several Se-dependent GSHpx isoforms (phospholipid, cytosolic, plasma, and gastrointestinal) as well as other selenoproteins (three iodothyronine 5′-deiodinases (types I, II and III), two thioredoxin reductases [TRs], and four other selenoproteins [in plasma (P), muscle (W), liver, and prostate]) [65].

The primary role for GSHpx is to defend against oxidative stress by catalyzing the reduction of H_2O_2 and organic hydroperoxides, which react with the selenol group of the active center of selenocysteine. The role of Se as a constituent of 5′-deiodinases (types I–III) is also of interest, because the thyroid gland produces quite large amounts of H_2O_2, which is used for the iodination of thyronine residues. The thyroid gland protects itself against oxidative damage by the activity of phospholipid and cytosolic GSHpx.

The peroxidase activity of GSHpx, together with the activity of TRs, is involved in a variety of key enzymes, transcription factors, and receptors. The discovery of the role of Se in TRs is of great interest because of the involvement of TRs in the modulation of redox-regulated signaling, including ribonucleotide reductase, prostaglandin and leukotriene synthesis, receptor-mediated phosphorylation cascades (ie, activation of NF-κβ), and even apoptosis [66,67].

The Se requirement of most animals is similar and is based on the maximization of GSHpx in plasma or red blood cells. Estimates of the Se requirement for kittens and adult cats [68,69] were determined to be 0.15 and 0.13 mg/kg, respectively, and for adult dogs [70], a diet containing Se at a rate of 0.10 mg/kg was recommended. Recommended allowances of Se in pet foods, which account for bioavailability, for dogs and cats are 0.35 and 0.40 mg/kg of diet, respectively [39].

Animal studies and clinical intervention trials with people have shown Se to be anticarcinogenic at intakes 5- to 10-fold greater than those recommended for recommended daily allowances or minimum requirements [65,67]. The following mechanisms have been proposed for the anticancer effects of Se: (1) antioxidant activity through GSHpx and TRs, (2) enhancement of immune functions, (3) alteration in the metabolism of carcinogens, (4) inhibition of tumor proliferation and enhancement of apoptosis, and (5) inhibition of angiogenesis [66].

Safe upper limits [SUL] for Se for most species are generally similar [71], approximately 2 mg/kg of food, although neither the Association of the American Feed Control Officials (AAFCO) [27] nor the NRC [39] has suggested an SUL of Se for the cat [68]. Compared with most other species, including dogs, cats display significantly higher Se concentrations in blood even when fed similar dietary Se intakes. It is unclear whether cats have a higher tolerance for Se, which may explain the inability to define an SUL, or whether other biomarkers or different forms of Se would have yielded more definitive limits. The AAFCO [27] suggests an SUL of Se of 2 mg/kg of diet for the dog [70].

Thiols: S-Adenosyl-L-Methionine, α-Lipoic Acid, and *N*-Acetylcysteine

Thiol metabolism has gained research momentum as the field of redox chemistry has matured. Thiols are capable of redox reactions similar to oxygen and have many metabolic correlates within the cell. Glutathione, S-adenosyl-L-methionine (SAMe), thioredoxin, and other sulfur-containing molecules have been shown to have important roles in metabolism and antioxidant defenses.

SAMe has been used successfully to treat acetaminophen toxicity in cats and dogs [72,73]. Administration of SAMe to clinically healthy cats has been shown to improve indices of redox status, as indicated by decreased red blood cell TBARS and increased hepatic glutathione disulfide (GSH) [74].

Lipoic acid is another thiol that may influence reduced glutathione content of cells. Administration of lipoic acid as a food additive resulted in increased ratios of white blood cells reduced to oxidized forms (GSH/GSSG) in dogs [75]. It has been shown that administration to cats has a prolonged elimination time compared with other species, however, and administration rates should be adjusted accordingly if used [11].

N-acetylcysteine has been shown to increase reduced glutathione in cats challenged by oral onion powder compared with controls [40]. *N*-acetylcysteine in combination with ascorbic acid has been shown to inhibit virus replication in feline immunodeficiency virus–infected cell lines [76]. Cysteine in combination with vitamin E was also shown to protect from acetaminophen-induced oxidative damage in cats [41].

Fruits and Vegetables

Fruits and vegetables are often rich in flavonoids, polyphenols, and anthocyanidins, ingredients that may all possess antioxidant properties. Exhaustive research of the effects of these ingredients in dogs and cats is not available, but a few studies have tried to evaluate some of the potential benefits of these additions to dietary regimens. Oral administration of a bioflavonoid complex reduced the amount of Heinz body anemia caused by acetaminophen administration in cats [77]. A combination of fruits and vegetables in a supplemented food was shown to increase selected flavonoids in the blood of aged dogs [78]. Although effective doses and safety are not well evaluated, it should be pointed out that administration of onion powder, which has purported antioxidant benefits in some species, to cats can result in Heinz body anemia, perhaps through increased oxidation [79].

Combination Therapies

In keeping with the idea that antioxidants work in networks, several studies have been designed to look at complex mixtures of antioxidants. Physiologic outcomes have varied; however, in general, positive results have been shown on immune function [80] and reduction in markers of antioxidant status or damage from oxidative stress [81–85]. In addition, long-term supplementation with a complex mixture of antioxidants has been shown to slow cognitive decline in aged dogs as well as to result in improved behavioral correlates in an

in-home study [86]. It is unknown in any of these studies what each component contributes to the final results, and this remains an area of research that needs development in the future.

SUMMARY

In summary, nutritional antioxidant research has developed dramatically in the quality and quantity of publications over the past several years. Certainly, this overview indicates positive benefits as well as many questions still to be researched. Nonetheless, it is anticipated that antioxidant benefits are likely to remain in the forefront of adjunctive therapies over the next several years as more detail is learned about mechanisms, interactions, and target benefits.

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