Iron

Iron is present in several enzymes and other proteins responsible for oxygen activation (oxidases and oxygenases), for electron transport (cytochromes) and for oxygen transport (haemoglobin, myoglobin). Because of the limited capacity of the body to excrete iron, iron homeostasis is maintained primarily by adjusting iron absorption. Iron in foods exists in two forms: 1) heme iron present in hemoglobin and myoglobin and 2) nonheme iron present in grains and plant sources.

Iron is transported by plasma and is taken up by the bone marrow for haemoglobin synthesis. Although a small amount of haemoglobin circulates in plasma, by far the greatest amount of plasma iron is complexed to the specific iron-binding β1-globulin transferrin. The degree of saturation of transferrin affects deposition of iron in liver stores and the supply of iron to red blood cell precursors. At saturation levels above 60%, much of the iron is deposited in the liver. Under normal conditions, only 30 to 40% of the transferrin is saturated; the remaining 60 to 70% represents an unbound or latent reserve.

Chronic blood loss eventually depletes iron reserves and causes a microcytic, hypochromic anaemia. The most common chronic blood loss in dogs and cats occurs with blood-sucking intestinal (hookworms) and external (fleas, ticks) parasites. Young puppies and kittens are especially vulnerable because of the low-iron content of milk[1,2].

Copper

In most species, copper can be absorbed in all segments of the GI tract; however, the small intestine is the major site of absorption. Although the biochemical mechanisms are not fully understood, there is good evidence that intestinal absorption of copper is regulated by the need of the animal and that metallothionein (a metal-binding protein) plays a key role in regulation. Copper appears to be absorbed by two mechanisms, one saturable, suggesting active transport at low dietary copper concentrations and the other unsaturable, suggesting simple diffusion at high dietary copper levels.

Most copper in plasma is bound to ceruloplasmin, a copper binding protein. Newly absorbed copper, however, may be transported from the intestine loosely bound to albumin or certain amino acids. In this form, the element is readily available to the liver and other tissues, in contrast to the much more tightly regulated distribution of ceruloplasmin-bound copper. This difference in availability may explain the tissue damage caused by copper accumulation in hepatotoxicosis seen in Bedlington terriers and people with Wilson's disease, in which the ceruloplasmin transport protein is lacking.

The liver is the central organ for copper metabolism. Hepatic concentrations reflect an animal's intake and copper status. Copper is excreted primarily through the feces. Most fecal copper is unabsorbed, but active excretion also occurs via the bile. Copper homeostasis is maintained primarily through absorption.

Dietary copper deficiency has been reported to occur in dogs and cats and thus is of practical concern[3]. Copper deficiency is associated with LAD. The primary cause of LAD may be copper deficiency, with zinc involved secondarily, or combined zinc and copper deficiencies[4].

Zinc

Zinc is a constituent or activator of more than 200 enzymes, so it is involved in a number of diverse physiologic functions. Some of zinc's primary functions include: 1) nucleic acid metabolism, 2) protein synthesis, 3) carbohydrate metabolism, 4) immunocompetence, 5) skin

and wound healing, 6) cell replication and differentiation, 7) growth and 8) reproduction. Zinc also interacts with hormone production, most notably testosterone, adrenal corticosteroids and insulin.

Zinc homeostasis is controlled through absorption and excretion. The mechanism and control of zinc absorption are still not fully understood. Zinc absorption occurs primarily in the duodenum, jejunum and ileum. Only small amounts are absorbed from the stomach. The liver is the primary organ involved in zinc metabolism. When hepatic zinc content is increased above normal levels, additional zinc is associated with metallothionein, a metal-binding protein thought to have a role in storage and detoxification of zinc, copper, cadmium and other metals. Zinc is excreted primarily in the feces as unabsorbed and endogenous zinc (pancreatic juice, bile, other digestive secretions). Excretion of endogenous zinc in feces varies according to the balance between true absorption and metabolic needs. Variable excretion is one of the primary mechanisms used to maintain zinc homeostasis. Thus, both absorption and excretion are important in regulating zinc balance.

Zinc deficiency is probably more of a practical concern with pet foods than is toxicity, because: 1) zinc is relatively nontoxic and 2) its availability is decreased by a number of factors (phytate, high dietary levels of calcium, phosphate, copper, iron, cadmium and chromium). The antagonistic effects of calcium are greatest when phytate is also present, resulting in the formation of a highly insoluble complex of calcium, phytate and zinc.

Signs of zinc deficiency have been reported to occur in dogs fed cereal-based dry foods (e.g., grains may contain significant concentrations of phytate), even when the zinc content of the food exceeded NRC minimum requirements[3].
Zinc-responsive dermatosis is an uncommon disease of dogs resulting from either an absolute or relative deficiency in zinc. Dermatological lesions are characterized by erythema, alopecia, scales, and crusts that primarily affect the head. Two forms of the disease exist: a familial form affecting Alaskan Malamutes and Siberian Huskies and a form that affects growing puppies fed zinc-deficient or oversupplemented diets. The history, clinical signs, and skin biopsy results are typically diagnostic. Life-long zinc supplementation is usually necessary in the familial form of the disease, although dietary correction alone may be curative in the second form. Lethal acrodermatitis is a rare inherited disorder of Bull Terriers that does not respond to zinc supplementation and is invariably fatal[5]. Serum zinc, iron, and copper levels are altered in canine leishmaniasis[6].

Vitamin A

Vitamin A is absorbed almost exclusively as the free alcohol retinol. Within mucosal cells, retinol is re-esterified mostly to palmitate and incorporated into the chylomicrons of the mucosa. Afterwards, it diffuses into lymph. Vitamin A is transported through the lymphatic system with low-density lipoprotein (LDL) to the liver, where it is deposited mainly in hepatocytes and stellate and parenchymal cells.

Some vitamin A derivatives are re-excreted into the intestinal lumen via the bile. This is true for much of retinoic acid and some retinol. The major vitamin A components of bile are vitamin A glucuronides, many of which are reabsorbed. Thus, enterohepatic circulation may provide an important means of conserving vitamin A. Although dogs and cats excrete vitamin A in urine, cats excrete a lesser amount.

When vitamin A is mobilized from the liver, stored vitamin A ester is hydrolyzed before it is released into the bloodstream. Vitamin A retinol is transported to tissues in the bloodstream by a specific transport protein called retinol-binding protein (RBP). RBP is synthesized and secreted by hepatic parenchymal cells.

In contrast to most other species, dogs and cats have a unique way of metabolizing vitamin A. Cats require preformed vitamin A because they lack the oxygenase enzyme necessary for β-carotene cleavage. In addition, studies have shown that cats and dogs do not depend on RBP to transport vitamin A in plasma. Cats and dogs transport vitamin A as retinyl esters (mostly retinyl stearate) bound to LDL and very low-density lipoprotein in amounts 10 to 50 times those of other mammals10. This is of interest because free circulating retinyl esters are a sign of hypervitaminosis A in almost all other animal species, including people.

Vitamin A is essential for a number of distinct biologic functions. It is necessary for normal vision, growth, reproduction, immune function and maintenance of healthy epithelial tissue. Vitamin A is also involved in the expression and regulation of many genes[7].
Vitamin A-responsive dermatosis in dogs is a rare condition characterized by an abnormality of cornification that occurs in adult dogs, predominantly Cocker Spaniels, but also in Labrador Retrievers and miniature Schnauzers. Owing to the breed-specific occurrence, a hereditary etiology is suspected, but the mode of inheritance is not known. Analysis of skin biopsy specimens combined with vitamin A supplementation is routinely used to confirm the diagnosis of a vitamin A-responsive dermatosis. The histopathologic hallmark of vitamin A-responsive dermatosis is orthokeratotic epidermal and predominantly follicular hyperkeratosis. Therapy consists of oral supplementation of 10,000 IU of vitamin A (retinol), once daily with food. A response and clinical resolution to therapy should be seen within 3 to 8 weeks. Treatment must be continued and is lifelong for most patients[8].

Vitamin B1 / Thiamine hydrochloride

Thiamine pyrophosphate (TPP) is the major coenzymatic form of thiamine and is required for only a small number of enzymatic reactions. Absorption takes place primarily in the jejunum by an active, carrier-mediated transport that also phosphorylates the vitamin. Passive diffusion becomes an important mode of absorption when dietary thiamin intake is high.
Absorbed thiamin is transported in erythrocytes, which contain free thiamin and its phosphorylated forms, and in plasma, which only contains free thiamin and its monophosphate form.
Tissues take up thiamin and may interconvert it between any of its four forms. The liver, heart and kidneys have the highest concentration of thiamin.

Most pet food manufacturers add additional sources of thiamine to compensate for thiamine lost through processing. However, despite best efforts, thiamine-deficient commercial pet foods sometimes are still produced. There is a clinical syndrome of thiamine deficiency in companion animals and it has been described in the literature[9].

Vitamin B2 / Riboflavin

Riboflavin is the precursor to a group of enzymatic cofactors called flavins. Flavins are used as coenzymes in about 50 enzymes in mammals. Most riboflavin found in food sources is in the form of free coenzyme derivatives that are not readily absorbed unless hydrolyzed, and covalently bound riboflavin that is not well used. The free flavin compounds are hydrolyzed before they are absorbed in the upper GI tract. A specialized transport system that is saturable and sodium dependent is necessary for absorption of flavins. After absorption, about 50% of

the riboflavin in blood is bound to albumin and the other half to globulins. Excess riboflavin in the body is eliminated largely as riboflavin via the kidneys.

Deficiency of riboflavin in dogs and cats is uncommon but may manifest as dermatitis, erythema, weight loss, cataracts, impaired reproduction, neurologic changes and anorexia[10].

Niacinamide

Niacin is the generic term used to describe compounds that exhibit biologic activity of nicotinamide. Two major forms of niacin are nicotinic acid and nicotinamide. Niacin, in its cofactor form, is essential to several physiologic reactions. Oxidoreductive reactions are the primary function, but others are significant in proper cell function. Generally, NAD/NADH is involved in catabolic reactions and transfers the reducing power (electrons) acquired from intermediary metabolites to the electron transport chain to ultimately produce adenosine triphosphate. Alternatively, NADP/NADPH is generally involved in biosynthetic reactions that transfer reducing power (electrons) to macromolecules such as fat, protein and carbohydrate.

Dietary niacin (nicotinic acid and nicotinamide) is absorbed readily through the gastric and small intestinal mucosa. Deficiency of niacin results in pellagra with its classic 4D signs: dermatitis, diarrhea, dementia and death. Clinical deficiency is uncommon in dogs because most commercial pet foods are supplemented with niacin. Cats, however, are more likely to develop signs of deficiency because of their strict requirement for niacin[11].

Pantothenic acid

Pantothenic acid is the trivial designation for dihydroxy-β, β- dimethylbutyryl-β-alanine. Only the dextrorotatory form of pantothenic acid has biologic activity. It occurs mainly in bound form, (i.e., coenzyme A [CoA] and acyl-carrier protein), in most foods and feedstuffs.

CoA is found in all tissues and is one of the most important coenzymes for metabolism. CoA plays a critical role in the tricarboxylic acid cycle for production of ATP from fat (glycerol and fatty acids), glucose and amino acids. CoA is also involved in the synthesis of fatty acids, steroid hormones and cholesterol. CoA is necessary for oxidation of fatty acids, pyruvate and ketoglutarate.

Absorption occurs via a saturable, sodium-dependent, energy requiring process. At high concentrations, simple diffusion occurs throughout the small intestine. Pantothenic acid is transported in the free acid form in plasma. Erythrocytes contain predominantly acetyl-CoA.

Naturally occurring deficiency of pantothenic acid is rare. Dogs with pantothenic acid deficiency have erratic appetites, depressed growth, fatty livers, decreased antibody response, hypocholesterolemia and coma, in later stages. Pantothenic acid-deficient cats developed fatty livers and became emaciated[12].

Vitamin B6 / Pyridoxine hydrochloride

Pyridoxine is also generally called vitamin B6. However, vitamin B6 is a generic descriptor for all 3-hydroxy-2-methylpyridine derivatives exhibiting the biologic activity of pyridoxine. The three naturally occurring forms of vitamin B6 are pyridoxal, pyridoxine and pyridoxamine. The biologically active forms of vitamin B6 are the coenzymes pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP). PLP is involved in most reactions of amino acid metabolism. PLP is also involved in the catabolism of glycogen and metabolism of lipids. As a coenzyme for decarboxylase enzymes, PLP functions in the synthesis of serotonin, epinephrine, norepinephrine and γ-aminobutyric acid (GABA). Pyridoxine is involved in vasodilatation through the production of histamine and is required in the pathway where niacin is produced

from tryptophan. Pyridoxine helps catalyze the synthesis of taurine from cysteine and participates with ascorbic acid and NAD in the synthesis of carnitine from the amino acid lysine. Pyridoxine is also involved with the synthesis of the heme precursor porphyrin (as a coenzyme for δ-aminolevulinate synthase).

Signs of vitamin B6 deficiency include anorexia, reduced growth, muscle weakness, neurologic signs, (e.g., hyperirritability and seizures), anemia, and irreversible kidney lesions. Oxalate crystalluria is also a notable sign in pyridoxine-deficient cats (NRC, 2006)[12].

Vitamin B12 / Cyanocobalamin

Vitamin B12 or cobalamin is the generic descriptor for all corrinoids exhibiting the biologic activity of cyanocobalamin. Vitamin B12 is the largest and most complex B vitamin and the only one to contain a metal ion, cobalt. The active forms of B12, 5'deoxyadenosylcobalamin and methylcobalamin, are very unstable. Substituted forms of vitamin B12 are much more stable and may be used as pharmaceutical supplements (cyanocobalamin, hydroxocobalamin, nitritocobalamin).

Vitamin B12 is important in one-carbon metabolism. In dogs and cats, methylcobalamin, which contains cobalt in the 1+ state, is a coenzyme for methionine synthase and 5'deoxyadenosylmethionine, which contains cobalt in the 2+ state is a coenzyme for methylmalonyl-CoA mutase. Vitamin B12 is required by the enzyme methionine synthase that removes a methyl group from methyl tetrahydrofolate (THF) to regenerate THF, which is needed for pyrimidine biosynthesis. This intimate relationship with folate may result in folate trapping in B12 deficiency and the resultant megaloblastic anemia of folate deficiency.

Vitamin B12 deficiency is very rare but may result in poor growth and neuropathies in dogs [13,14]. Because vitamin B12 is only made by microbes and found in animal tissue, long-term feeding of vegetarian diets may lead to vitamin B12 deficiency.

Vitamin E

Vitamin E is a term for a group of compounds with the biologic activity of α-tocopherol. In nature, there are eight isomeric forms of vitamin E, four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ). The most biopotent form of vitamin E is α-tocopherol. The relative biopotencies of vitamin E isomers are as follows: α >β >δ >γ. Also, tocopherols are generally more available than tocotrienols [15].

Vitamin E works in conjunction with glutathione peroxidase to protect cells against the adverse effects of reactive oxygen and other free radicals that initiate the oxidation of polyunsaturated membrane phospholipids. Vitamin E in cellular and subcellular membranes is the first line of defense against peroxidation of vital phospholipids.

In addition, vitamin E is important for normal reproduction and is involved in modulating cellular signaling, regulating gene transcription, modulating immune function and inducing apoptosis[16].

The clinical manifestations of vitamin E deficiency vary markedly between species. In general, however, the neuromuscular, vascular and reproductive systems are affected most commonly. Signs of vitamin E deficiency are mostly attributed to membrane dysfunction as a result of the oxidative degradation of polyunsaturated membrane phospholipids and disruption of other critical cellular processes. Clinical findings of vitamin E deficiency in dogs include degenerative skeletal muscle disease associated with muscle weakness, degeneration of testicular germinal epithelium and impaired spermatogenesis, failure of gestation, brown

pigmentation (lipofuscinosis) of intestinal smooth muscle and decreased plasma tocopherol concentrations. In cats, deficiency signs include steatitis, focal interstitial myocarditis, focal myositis of skeletal muscle and periportal mononuclear infiltration in the liver[17].

Vitamin D3

The primary function of vitamin D is to enhance intestinal absorption and mobilization, as well as retention and bone deposition of calcium and phosphorus. This function is manifested through its active form of 1,25-(OH)2-D3 as a hormone that binds to the nuclear 1,25-(OH)2-D3 receptor (VDR) in many types of cells. The active vitamin D3 also has a direct effect on Ca2+ channels located on the plasma membrane.

Vitamin D is distributed relatively evenly among the various tissues where it resides in lipid depots. Vitamin D can be found in adipose, kidneys, liver, lungs, aorta and heart. The primary circulating form of vitamin D is the parent vitamin D (~50%), with the next most abundant form (i.e., 25-OH-D3 [also called calcidiol]) accounting for approximately 20% of the total. In mammals, both vitamin D2 (ergocalciferol) and D3 (cholecalciferol) are not the active form of vitamin D. They are activated in the body by hydroxylation to 25-OH-D3 first in the liver and again to 1,25- (OH)2-D3 (also called calcitriol) in the kidneys. Vitamin D2 is less efficiently used than vitamin D3 in cats[18]. There is a lack of controlled, well-powered studies in dogs, but vitamins D2 and D3 appear to have a similar potency in this species[19].

Signs of vitamin D deficiency are frequently confounded by a simultaneous deficiency or imbalance of calcium and phosphorus. Clinical signs generally include rickets (young animals), enlarged costochondral junctions, osteomalacia (adult animals), osteoporosis (adult animals) and decreased serum calcium and inorganic phosphorus concentrations[20].

Daily oral calcitriol at low doses is safe and effective in the control of renal secondary hyperparathyroidism in dogs and cats. The salutary effects on the dog's or cat's sense of well-being, appetite, activity, strength, and lifespan as reported by the veterinarians[21] were attributed primarily to keeping PTH levels below a toxic threshold. Additionally, some of the benefits achieved by calcitriol were likely a direct consequence of calcitriol interacting with the vitamin D receptor in a wide variety of tissues throughout the body.

References

1. http://www.markmorrisinstitute.org/sacn5_download.html
2. Bohn AA. Diagnosis of Disorders of Iron Metabolism in Dogs and Cats. Clin Lab Med. 2015 Sep;35(3):579-90. doi: 10.1016/j.cll.2015.05.007. PMID: 26297405.
3. Morris JG, Rogers QR. Assessment of the nutritional adequacy of pet food through the life cycle. Journal of Nutrition 1994; 124: 2520S-2534S.
4. Uchida Y, Moon-Fanelli AA, Dodman NH, Clegg MS, Keen CL. Serum concentrations of zinc and copper in bull terriers with lethal acrodermatitis and tail-chasing behavior. Am J Vet Res. 1997 Aug;58(8):808-10. PMID: 9256960.
5. Colombini S. Canine zinc-responsive dermatosis. Vet Clin North Am Small Anim Pract. 1999 Nov;29(6):1373-83. doi: 10.1016/s0195-5616(99)50133-2. PMID: 10563006.
6. Pasa S, Kargin F, Bildik A, Seyrek K, Ozbel Y, Ozensoy S. Serum and hair levels of zinc and other elements in dogs with visceral leishmaniasis. Biol Trace Elem Res. 2003 Aug;94(2):141-7. doi: 10.1385/BTER:94:2:141. PMID: 12958405.

7. McClintick JN, Crabb DW, Tian H, et al. Global effects of vitamin A deficiency on gene expression in rat liver: Evidence for hypoandrogenism. Journal of Nutritional Biochemistry October 5, 2005.
8. Hensel, P. (2010). Nutrition and skin diseases in veterinary medicine. Clinics in Dermatology, 28(6), 686–693. doi:10.1016/j.clindermatol.2010.03.031
9. Markovich, J. E., Heinze, C. R., & Freeman, L. M. (2013). Thiamine deficiency in dogs and cats. Journal of the American Veterinary Medical Association, 243(5), 649–656. doi:10.2460/javma.243.5.649
10. (National Research Council). Nutrient Requirements of Dogs. Washington, DC: National Academies Press, 1985.
11. MacDonald ML, Rogers QR, Morris JG. Nutrition of the domestic cat, a mammalian carnivore. Annu Rev Nutr. 1984;4:521-62. doi: 10.1146/annurev.nu.04.070184.002513. PMID: 6380542.
12. (National Research Council). Nutrient Requirements of Dogs and Cats. Washington, DC: National Academies Press, 2006
13. Fyfe JC, Jezyk PF, Giger U, et al. Inherited selective malabsorption of vitamin B12 in giant schnauzers. Journal of the American Animal Hospital Association 1989; 25: 533-539.
14. Vaden SL, Wood PA, Ledley FD, et al. Cobalamin deficiency associated with methylmalonic acidemia in a cat. Journal of the American Veterinary Medical Association 1992; 200: 1101-1103.
15. Combs GF Jr, ed. The Vitamins, Fundamental Aspects in Nutrition and Health, 2nd ed. San Diego, CA: Academic Press Inc, 1998.
16. Brigelius-Flohe R, Kelly FJ, Salonen JT, et al. The European perspective on vitamin E: Current knowledge and future research. American Journal of Clinical Nutrition 2002; 76: 703-716.
17. Koutinas AF, Miller WH, Kritsepi M, et al. Pansteatitis (steatitis,"yellow fat disease") in the cat: A review article and report of four spontaneous cases. Veterinary Dermatology 1993; 3: 101-106
18. Morris JG. Cats discriminate between cholecalciferol and ergocalciferol. Journal of Animal Physiology and Animal Nutrition 2002; 86: 229-238.
19. Delaney S.J. Serum ionized calcium, 25-hydroxyvitamin D, and parathyroid hormone in two dogs fed a homemade diet fortified with vitamin D2. In: 2015 Annual AAVN Symposium Order of Abstracts J Anim Physiol Anim Nutr, 99 (2015), pp. 810-824
20. Uhl EW. The pathology of vitamin D deficiency in domesticated animals: An evolutionary and comparative overview. Int J Paleopathol. 2018 Dec;23:100-109. doi: 10.1016/j.ijpp.2018.03.001. Epub 2018 Mar 13. PMID: 29544996.
21. Nagode LA, Chew DJ, Podell M. Benefits of calcitriol therapy and serum phosphorus control in dogs and cats with chronic renal failure. Both are essential to prevent of suppress toxic hyperparathyroidism. Vet Clin North Am Small Anim Pract. 1996 Nov;26(6):1293-330. doi: 10.1016/s0195-5616(96)50130-0. PMID: 8911021.